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4756

Lymph Reactions Following Bulbus Pressure in Dogs.

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During the course of studies on the lymph of normal dogs under various conditions we have observed that practically every insult is followed by rhythmic changes of organ activity as indicated by the chemistry of the lymph,[1] and the leucocyte count.[2] We have called attention to the probability of a balance between the peripheral portion of the organism and the splanchnic region,[3] the one being more active when the other is at rest. The leucocyte picture in particular gives us most striking evidence of this relation.[4]

Incidental to these observations we have carried out a series of experiments, with uniform technique, to study the lymph changes following bulbus pressure (oculo-cardiac reflex), the pressure being applied with sufficient intensity to perceptibly slow the cardiac rate.

In a chart of such an experiment previously published[5] we have

[1] Petersen, W. F., Müller, E. F., and Boikan, Wm., *J. Infect. Dis.*, 1927, xli, 405; *Z. f. d. ges. Exp. Med.*, 1928, lx, 336.

[2] Müller, E. F., and Hoelscher, R., *Klin. Wschr.*, 1929, viii, 1027.

[3] Petersen, W. F., and Müller, E. F., *Arch. Int. Med.*, 1927, xl, 575.

[4] Müller, E. F., and Petersen, W. F., *Klin. Wschr.*, 1926, v, 53.

[5] Petersen, W. F., and Levinson, S. A., *Arch. Path.* 9, 1930, ii, 405.

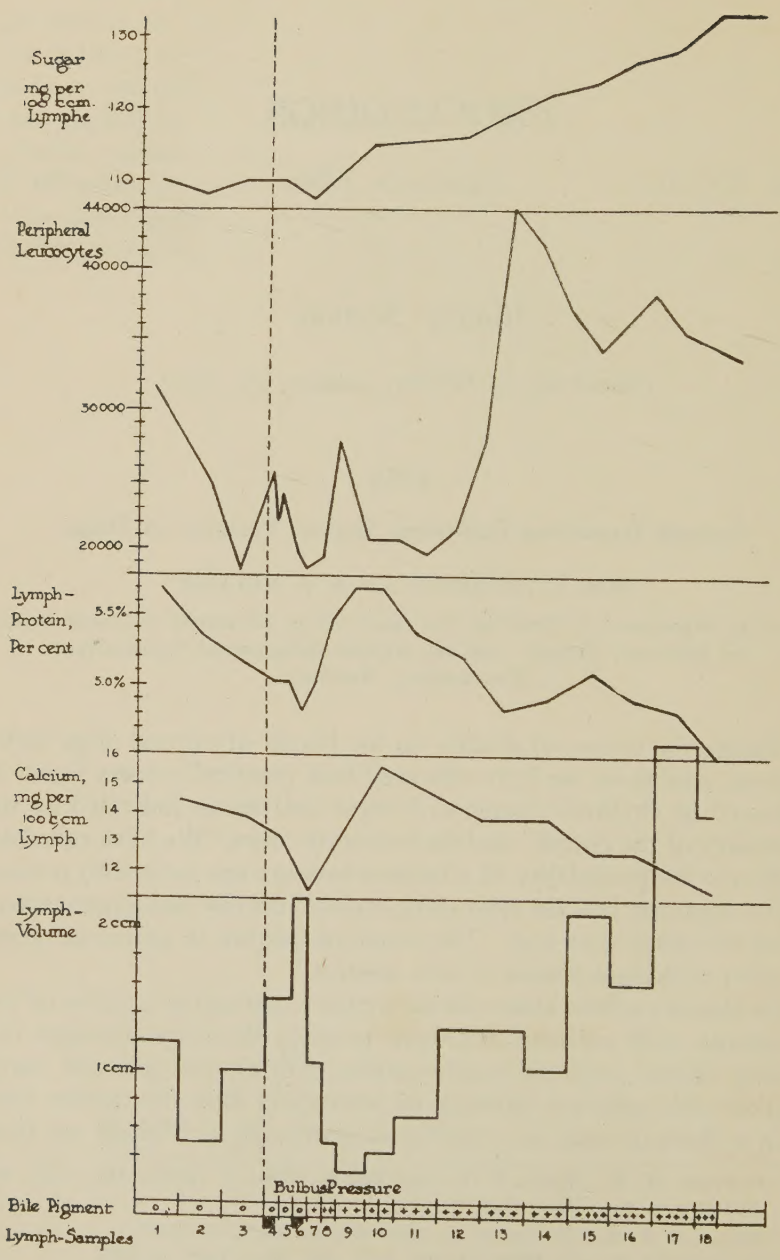

CHART No. 1.

Lymph changes and peripheral leucocyte count following eye pressure in dogs.

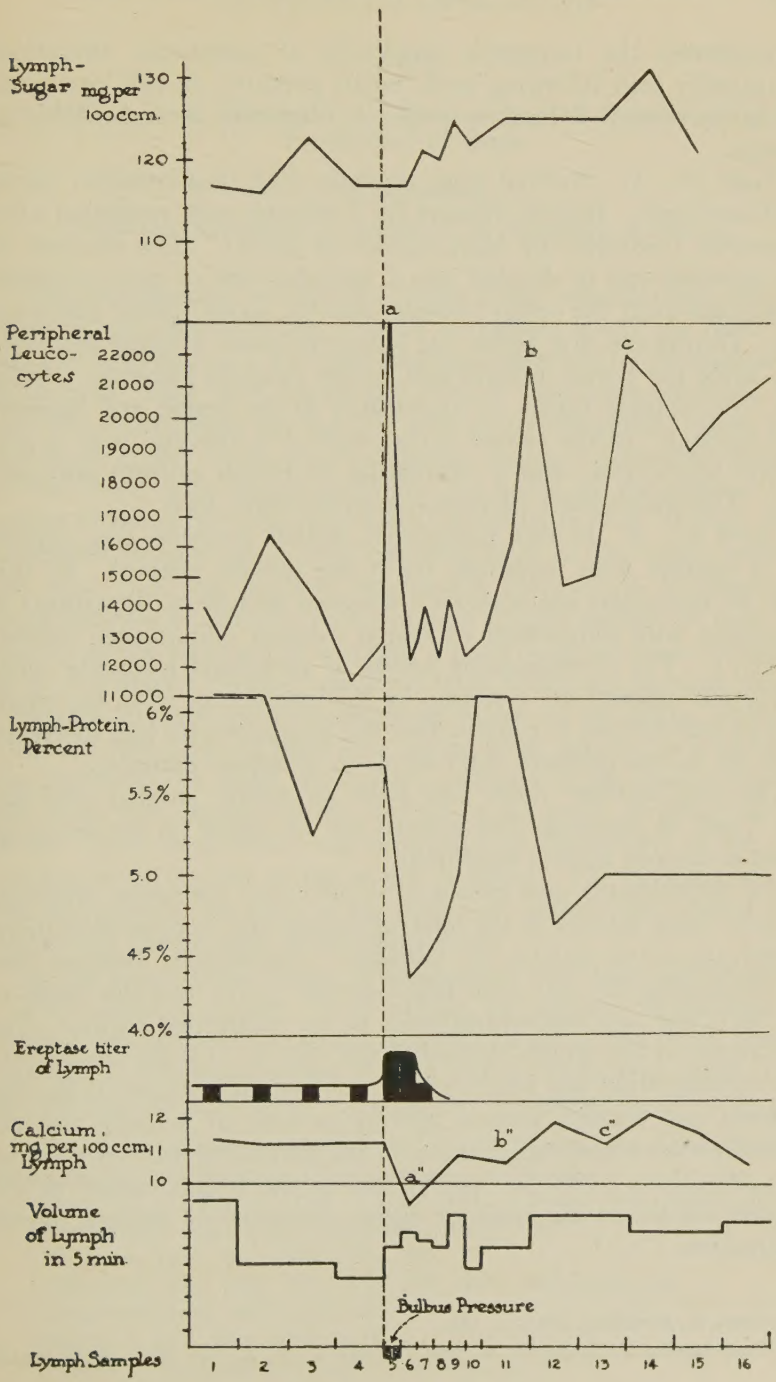

CHART No. 2.

Lymph changes and peripheral leucocyte count following eye pressure in dogs.

demonstrated the increasing amplitude of autonomic alterations occasionally seen following such bulbus pressure; in this communication we present 2 further graphs to illustrate several additional changes.

Chart No. 1. Normal dog, thoracic duct incannulation under local anesthetic. Bulbus pressure for 4 minutes with repetition after 5 minutes (indicated by black blocks on chart). The reaction of the organism can be divided into 2 periods—one of approximately 75 minutes after the ocular pressure and the second phase following this. During the first phase, the leucocyte count remains relatively low while the lymph protein and lymph calcium increases. With this, bile pigment makes its appearance in the lymph and becomes well marked. In the second period there is a reversal with a peripheral leucocytosis and a diminution of lymph calcium and protein. The bile pigment continues at an unaltered level.

Chart No. 2. In this experiment, bulbus pressure was applied for 2 minutes with repetition for a one minute interval. In this case, an immediate leucocytosis (increased peripheral dilatation) is associated with diminution of lymph calcium (diminished cellular activity). The corresponding waves of peripheral leucocyte count and lymph calcium are indicated by the letters a, b, c, on the chart. It is of interest too, to observe that the lymph erepsin had increased after the bulbus pressure, later seems to disappear entirely.

The oculo-cardiac reflex has been repeatedly studied clinically and Tinel[6] in particular has pointed out the effect on the leucocyte count in various clinical conditions.

Our experiments give evidence of profound alteration produced by such reflex activity in the internal organs and indicate alterations of the autonomic balance so initiated. Danielopolu[7] believes that we are dealing not only with impulses that travel over the vagus to the heart but over the sympathetics to the splanchnic region. The alterations of the lymph consisting not only of changes in the protein concentration and calcium level but also of changes in the bile pigment and enzyme content portray periods of activity and depression with associated changes in the peripheral leucocyte count. The alterations which we have found in the lymph cannot be explained wholly on the basis of either voluntary or smooth muscle contraction.

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[6] Tinel, J., *Medicine*, 1923, iv, 463.

[7] Danielopolu, D., Radovici, A., and Carniol, A., *C. R. Soc. Biol.*, 1922, lxxxvi, 637.

4757

Influence of Urinary Tract Mucosa on the Experimental Formation of Bone.

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The experimental formation of bone in the urinary tract was first observed by Sacerdotti and Frattin[1] in rabbits where the renal vessels had been ligated. This has also been observed by Poscharissky,[2] Maximow,[3] Liek[4] and Asami and Dock.[5] The bone occurs in thin lamellae immediately beneath the mucosa of the pelvis. Bone does not occur if the ureter also is ligated. Pearce,[6] following excision of the lower pole (approximately one-half) of the kidney with suture of the defect, observed bone formation in 6 of 19 dogs. Bone developed in connection with epithelial buds of the pelvic mucosa. He says "for this peculiar localization there is no explanation." In application of phenol (95%) and an electric current of high frequency to the renal pelvis of dogs, I have observed bone formation in 3 of 5 dogs. Strauss[7] observed bone formation beneath the regenerated ureteral mucosa, lining the lumen of the graft. Neuhof[8] observed formation of bone in a fascial patch sutured in a defect of the bladder in 18 dogs. Invariably confined to the fascia replacing the defect and situated in the surface of the graft bordering on the urine. This observation was confirmed by Phemister[9] in the dog, but not in the bladder of the rabbit or sheep. The explanations for the development of bone in the ureter or bladder were based on a metaplasia due to some factor in the urine.

In my experiments, auto-transplantation was done throughout.

Experiment I: 3 dogs. Both ureters transplanted to the skin, the bladder was emptied and fascia from the sheath of the *rectus abdominis* muscle was transplanted to fill a circular operative defect 3 cm. in diameter in the dome of the bladder. Examination 24 to 47 days

* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

1 Sacerdotti and Frattin, *Arch. f. Path. Anat. u. Phys.*, 1902, clxviii, 431.

2 Poscharissky, *Beitr. Path. Anat.*, 1905, xxxviii, 135.

3 Maximow, *Anat. Anz.*, 1906, xxviii, 609.

4 Liek, *Arch. Klin. Chir.*, 1906, lxxx, 279; *Ibid.*, 1908, lxxxv, 118.

5 Asami and Dock, 1920, xxxii, 745.

6 Pearce, *J. Med. Res.*, 1909, xx, 53.

7 Strauss, *Surg. Gynec. and Obst.*, 1914, xviii, 78.

8 Neuhof, *Surg. Gynec. and Obst.*, 1917, xxiv, 383.

9 Phemister, *Ann. of Surg.*, 1923, lxxviii, 239.

later showed bone formation in the fascial patch and confined to it. Microscopically, it was true lamellar, membranous bone containing Haversian systems. There were islands of bladder mucosa cells in the depths of the transplant and some islands of bone: whenever bone occurred, mucosal cells were closely adjacent.

Experiment II: 9 dogs. Portions of (a) bladder mucous membrane alone, and (b) entire bladder wall were excised and implanted in (c) *fascia lata* of thigh (d) sheath of rectus muscle. The results were similar without regard to the combination. An epithelial lined cyst formed at the 7th to 9th day. The adjacent connective tissue became edematous with large deeply staining nuclei in places at the 16th day, and bone developed surrounding a portion of the cyst, never more than one-half the circumference from the 20th day on. The epithelium had been sewed in so that its free surface faced towards the skin. The bone was always situated around that portion of the cyst nearest the skin. Was it due to gravity?

Experiment III: 8 dogs. Transplants of bladder mucosa and entire bladder wall were sewed in rectus sheath in such a way that (a) the free surface of the mucous membrane faced in the direction of the peritoneal cavity in some cases and in others (b) in the direction of the skin. In the cysts developing in (a) the bone faced the peritoneal cavity and in (b) towards the skin. The development of bone was always adjacent to the new formed mucosal portion of the cyst.

Experiment IV: 5 dogs. Scrapings of mucosa of the urinary bladder deposited in rectus sheath, yielded a cyst with bone completely surrounding it.

Experiment V: Transplantation of ureter, renal pelvis and hilus portion of calyx into connective tissue of abdominal wall gives precisely the same result as bladder. Similar transplantation of renal cortex, medulla or papilla portion of calyx fails to produce bone.

Experiment VI: Transplantation of bladder mucosa in (1) liver; (2) spleen; (3) kidney; produced a cyst lined with transitional epithelium: the wall of the cyst is surrounded by connective tissue. No bone formed. Transplantation of bladder mucosa in the lung produced a chronic lung abscess in 3 dogs, but no bone.

Experiment VII: Transplantation of gall bladder wall, adrenal, stomach wall, small intestine, colon and prostate into connective tissue of abdominal wall failed to produce bone although the graft survived in each case.

Experiment VIII: Transplantation of bladder mucosa in subcutaneous fat of abdominal wall, and in striated muscle produced cysts

surrounded in part by bone as in Experiment III. Insertion of urinary bladder mucosa into the knee joint was followed by attachment of the mucosa to the synovial membrane and formation of a plaque of bone here.

Experiment IX: Clean excision of the mucous membrane of the urinary bladder with the exception of the trigone and a narrow strip around each ureter and the urethra, was followed by regeneration of the mucosa over the denuded parietes, but no bone formed at the end of 90 days.

Experiment X: Transplantation of bladder mucosa to the sheath of the abdominal rectus muscle in 6 rabbits produced epithelial lined cysts surrounded by connective tissue but no bone as early as 60 days. In one rabbit at 93 days, there was a deposition of bone.

Conclusion: The influence of epithelium on connective tissue in certain places causing the formation of bone is demonstrated for the first time.

4758

Bronchial Fistula. A Method of Experimental Production.*

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Bronchial fistula, a not uncommon complication following certain operations and disease conditions in man, has been found somewhat difficult to produce experimentally. This fact is substantiated by the work of Pool and Garlock,[1] and others, who found the production of bronchial fistula in dogs attended with great technical difficulties. Their method consisted of resecting a piece of rib and suturing the underlying lung lobe to the very thin parietal pleura over an area 1.25 inches in diameter. At a second stage operation 2 weeks later, the lung parenchyma found adherent to the chest wall was entered by careful blunt dissection until a fair sized bronchus was located. This was then opened and the skin margins sutured to the opening in the bronchus. They were unable to retain this opening, however, without almost daily cauterization, as it tended to close spontaneously.

* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Pool, E. H., and Garlock, J. H., *Ann. Surg.*, 1929, xc, 213.

Having no knowledge of the above procedure, we first attempted to produce a bronchial fistula in much the same manner, *viz:* At the first operation a piece of rib was resected and the underlying lung sutured to the parietal pleura and periosteum over an area 1.5 inches in diameter. At a second operation 2 weeks later, the area enclosed by the suture was entered with the actual cautery heated to a dull red until an area 1 cm. in diameter and about 1 inch in depth was burned in the lobe. The skin was sutured around the burned area. A bronchial fistula resulted; however it was very deep seated and also quite small so that this method was cast aside and the following procedure adopted. At the first operation, 2.5 inches of the right 6th rib were removed sub-periosteally at about its mid-point. Under positive-pressure ether anesthesia[2] the thoracic cavity was entered through a longitudinal opening in the rib-bed about 2 inches in length. The right middle lobe was then located and about 75% of it delivered outside the thoracic wall. By using a fine curved needle and a double fine silk suture the lobe was secured to the surrounding pleura, periosteum and intercostal muscles by a single row of continuous sutures. The tissues were then closed in layers over the lung lobe, using catgut for muscle and subcutaneous tissues and linen for the skin. Two weeks later the second operation was performed. The scar of the former operation was excised and the lung lobe outside the thoracic wall located and separated by blunt dissection, from the surrounding tissues down to the row of silk sutures. Firm adhesions were found between the lung lobe and chest wall. The lobe was then amputated close to the chest wall with the actual cautery heated to a dull red. Hemostasis was easily obtained with the cautery. The largest air passage was then located and a rubber tube about 0.25 inch in diameter inserted into it for a distance of 1 or 2 cm. The tissues were then closed in layers over the amputation stump and around the tube, catgut being used beneath the skin, and linen for the latter. A safety pin was placed in the rubber tube and a sterile dressing and body cast applied. Daily dressings were applied through a window cut in the cast, at which time the tube was cleansed and the wound flushed with warm dilute iodine solution. However, the fistula always became infected. The cast was taken off and the tube removed in 2 to 3 weeks.

Results: Of 12 dogs treated in the above manner, 3 died on the 1st or 2nd day following the 2nd stage operation; one presumably from shock, another from hemorrhage due to trauma of the wound

[2] Livingstone, H., and Hrdina, L. S., "An Apparatus for the Administration of Positive Pressure Anesthesia." (Unpublished work.)

by clawing and a third from pneumothorax due to false passage of the tube into the lung parenchyma with subsequent perforation into the pleural cavity. The remaining 9 are healthy, lively animals with a bronchial fistula. If the fistula remained infected there has been no tendency toward closure up to the present time, a period of 4 months. However, if the infection clears up, as it has done in some dogs, there occurs a "crusting" at the broncho-cutaneous junction, with closure of the fistula by granulation tissue.

In some recent experimental work on bronchial injury and repair,[3] it was found that a bronchus 0.25 to 0.5 inch in diameter could be completely stenosed by repeated thermal cauterization. A complete stenosis was also obtained within 2 weeks with one application of a 75% silver nitrate solution. In view of these findings, it is reasonable to believe that a persistent bronchial fistula may be permanently closed by one of these methods.

4759

Changes in Humoral Immunity Occurring During the Early Stages of Experimental Pneumococcus Infection.*

EDWARD E. TERRELL. (Introduced by O. H. Robertson.)

From the Department of Medicine of the University of Chicago.

A study was made of the changes in humoral immunity occurring during the early phases of experimental pneumococcus infection in the dog and cat, employing the methods devised by Robertson and Sia[1] for demonstrating the presence of anti-pneumococcus properties in the serum of animals naturally resistant to this microorganism. It was found that with a generalized and overwhelming infection accompanied by early blood invasion, there was a prompt and rapid decrease in the concentration of natural humoral immune bodies which frequently disappeared entirely by the time of death. This same early diminution of humoral immune substances, opsonins, agglutinins, and pneumococcidal promoting bodies was observed to occur in animals recovering from a moderately severe generalized infection with the difference that the concentration of immune bodies

[3] Adams, W. E., and Livingstone, H., *Ann. Surg.* (in press).

* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Robertson, O. H., and Sia, R. H. P., *J. Exp. Med.*, 1924, xxxix, 219; 1927, xlvi, 239.

began to rise coincident with the onset of recovery. The decrease in concentration of humoral immune substances during a severe generalized infection appeared to be due to the combination of "S" substance with the normal immune bodies.

When the pneumococcus infection was more localized as in the case of true lobar pneumonia, a quite different sequence of events was observed to occur. Several animals in which extensive lobar pneumonia was produced showed well marked concentration of humoral immune bodies in the blood throughout the course of a fatally terminating infection.

These findings would suggest that after the inception of pneumococcus infection in the dog and cat the chief function of natural anti-pneumococcus substances in the blood is to limit or prevent blood invasion. In the presence of localized pneumococcus infection, the persistence of these circulating antibodies appears to have little effect either in preventing the spread of the process or determining the outcome of the disease.

4760

Ultraviolet "Point Radiation" Focussed Through a Quartz Rod and Effect on Fundulus Heart Beat.*

MARIE A. HINRICHS.

From the Hull Physiological Laboratory, University of Chicago.

In a series of papers, the first of which appeared in 1912, Tschachotin[1] described a method of focussing ultraviolet radiation into a cell by means of a system of quartz oculars and objectives in a microscope. The following experiments make use of a principle well-known to physicists, and recently demonstrated by Wolf[2] to be of value in biological experiment, namely that ultraviolet radiation will follow a quartz rod, be it straight or bent, because of internal reflection. If the rod is drawn out to a fine point, radiation effects may be limited to a small area.

As will be seen from the accompanying sketch, a quartz rod was placed at right angles and close to the burner of a mercury-vapor lamp. A shutter control was interposed between the rod and the

* Study supported in part by a grant from the Radiation Fund of the National Research Council.

[1] Tschachotin, S., *Biol. Centralblatt*, 1912, xxxii, 623.

[2] Wolf, E., *The Collecting Net* (Woods Hole), 1929, iii, 20.

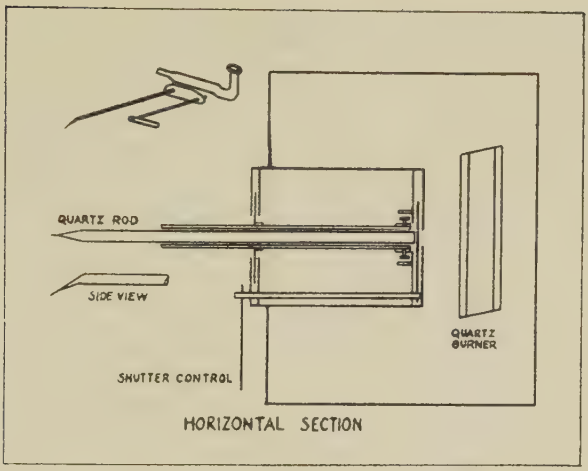

FIG. 1.

burner. The length of the rod was 29 cm. with a 45° bend at 1.0 cm. from the tip. (In order to minimize "leakage", the area just above the tip was painted with opaque paint, or in some cases covered with a glass cap.) The rod was held in place by a universal joint, as indicated, and was freely movable in all directions. A very small change of position at the fixed end afforded considerable range of movement at the free end. A movable stage, not indicated in the diagram, aided in focussing the radiation on the desired spot on the heart.

The experimental material was young *Fundulus heteroclitus,* a small salt-water minnow. Shortly after hatching, the body wall is still transparent so that heart movements can be studied through it. The animals were quieted with a sea-water solution of chloretone, and turned ventral side up. In each case, a record was made of the rate of heart beat before and after radiation. The various regions of the heart (Sinus, Atrium, Bulbus) were radiated at different times, and the effect of such exposures on both the general heart beat and on the separate regions of the heart is indicated in the following tables.

It will be seen that, at room temperature, short exposures in the sinus region increase the rate of heart beat, while longer exposures decrease it. Exposures in the bulbus region produce an increase in every instance. When the heart beat has been previously slowed by a decrease in temperature, longer exposures in the sinus region produce an increase in rate, whereas, at room temperatures, such exposure usually produce a decrease in rate of heart beat. (See Table I.)

TABLE I. *Effect on General Heart Beat.*

Original rate Beats/min.	New rate Beats/min.	% Gain	Exposures made on Sinus (Figures indicate duration of exp. in minutes.)	Atrium	Bulbus
			min.	min.	min.
112	141	26	0.5		
121	137	13	3.5		
151	142	–6	4.5		
126	122	–3	6.0		
151	149	–1	9.0		
161	177	10	9.0		
126	122	–3	10.0		
* 36	41	13	11.0		
* 47	63	33	13.0		
151	162	7		5.0	
121	139	15	0.5		0.5
111	128	15			1.0
151	157	4			4.5
111	123	11			9.0
121	174	21	0.5		9.5

* Kept on ice throughout experiment.

A study of regional effects (Table II) showed that exposures of from 1 to 5 minutes, in the region of the sinus, were generally stimulative in all regions of the heart. A 12-minute exposure was slightly depressing. Two-minute exposures made in the sinus region (in the cold) produced a stimulation in all regions, the average stim-

TABLE II. *Regional Effect on Heart Beat.*

Original Rate*	Region Exposed	Effect on rate of (% Gain) Sinus	Atrium	Bulbus
† 47	sinus 0.5 min.	+ 9%		+ 1%
116	1.0	+18		+18
† 47	2.0	— 6	+ 1%	+ 2
† 47	2.0	— 5	+16	+10
† 47	2.0	+ 8	+12	+ 8
† 47	2.0	+ 8	+13	+ 4
† 53	2.0	+25		+23
† 53	2.0	+32		+30
‡ 60	2.0	+ 6		+ 8
116	2.0	+16		+16
116	5.0	+17		+11
126	12.0	— 9		— 6
† 47	bulbus 1.0 min.	+19%		+21%
116	2.0	+12		+18
† 53	2.0	+17		+27
‡ 60	2.0	+13		+18

* Rate measured as beats per minute.
† Kept on ice. ‡ Excess of chloretone added.

ulation being greatest in the bulbus region, less in the atrium, and least of all in the sinus region. Similar effects are obtained by applying radiation in the bulbus region.

It was found that the average gain in rate of heart beat following radiation was noticeably greater when the heat had been previously slowed through a lowering of the temperature of the sea-water in which the eggs were kept. Table III shows a record of such a series of exposures. The average Q_{10} was slightly greater for nonradiated than for radiated hearts.

TABLE III. *Effect of the Combined Action of Low Temperature and Ultraviolet Radiation on the Rate of Heart Beat.*

Temp.	20 degrees C.			10 degrees C.			
Normal	Radiated	% gain in rate	Q_{10}	Normal	Radiated	% gain in rate	Q_{10}
*109	*155	42	2.14	*51	*62	21	2.50
131	142	8	2.57	51	66	29	2.15
137	136	− 1	2.80	49	69	41	1.97
112	150	34	2.00	56	63	12	2.38
100	104	4	2.27	44	52	18	2.00
119	127	7	2.48	48	50	4	2.54
112	150	34	2.43	46	76	65	1.97
128	132	3	3.56	36	43	19	3.07

* Figures in columns so marked represent number of beats per minute.
Average % gain in rate at 20 degrees—16⅜%.
Average % gain in rate at 10 degrees—26⅛%.
Average Q_{10} normal hearts—2.53.
Average Q_{10} radiated hearts—2.32.

The effectiveness of applying radiation to a restricted area by such a method as this depends on the degree of local change in physiological activity induced in that area, and should find further application in embryological and physiological study. A series of experiments involving "point" radiation studies on the chick embryo is now under way.

I wish here to express my thanks to Dr. R. S. Lillie for his helpful suggestions during the study of this problem, to Miss Ida Genther (of Washington University, St. Louis) for assistance in the laboratory, and to Mr. J. J. Ryan for designing and constructing the apparatus.

4761

The Permeability of the Synovial Membranes.

GEORGE M. CURTIS AND A. BRUNSCHWIG.

From the Department of Surgery, University of Chicago.

Little is known of the physiology of the synovial membranes, particularly regarding their permeability. As a consequence, it is difficult to understand the effusions occurring in joint cavities, or the accumulations of fluid in tendon sheaths and in bursae, consequent to trauma or disease. The knee joint is usually studied since it is easily accessible. In addition, it possesses the most extensive synovial membrane in the body. Diffusible constituents pass readily from the blood into the synovial fluid. In fact Crouter, Cajori and Pemberton[1] have shown that their concentration in synovial fluid can be readily changed by inducing corresponding changes in the blood. Fisher[2] observed that potassium iodide, Berlin Blue, and colloidal silver were absorbed from the knee joints of rabbits. He demonstrated iodide in the urine but did not determine in this manner its rate of absorption from the synovial cavity.

Using the method of Dandy and Rowntree,[3] we have studied the permeability of the synovial membrane to phenolsulphonephthalein in 5 male patients with effusion in the knee joint. In 4 this was traumatic in origin; in one it was due to chronic infection. With the patient at rest during the entire procedure, a soft rubber catheter a demeure was first introduced into the bladder. Under strict asepsis and local anesthesia, a large bored needle was then inserted into the joint cavity through the upper and outer portion of the joint capsule. As much as possible of the effusion fluid was aspirated without undue manipulation. This was cultured, injected into guinea pigs and analyzed chemically. The usual amount, 6 mg. in 1 cc., of P.S.P. was then injected into the joint cavity through the aspirating needle. Its appearance time was noted in the urine draining from the catheter, and the catheter was then removed. The urine was voided at the end of the first and second hour, and the amount of dye excreted determined colorimetrically.

The results are presented in Table I. In one patient, with chronic multiple arthritis of 9 years duration, *Streptococcus viridans* was isolated from the synovial fluid. The remaining fluids were sterile

[1] Cajori, Crouter and Pemberton, *Arch. Int. Med.*, 1926, xxvii, 92.

[2] Fisher, T., *Lancet*, 1923, ccv, 541.

[3] Dandy and Rowntree, *Annals of Surgery*, 1914, lix, 587.

to culture and guinea pig inoculation. Chronic infection, in this one instance, did not appear to decrease the permeability; if anything, it increased it. The catheter appearance time of the P.S.P. varied between 9 and 13½ minutes, averaging 11½ minutes in 6 tests. This is slower than that following the usual intravenous or even intramuscular administration. The amount excreted is within the normal range, save in one case in which it is low. In all 5 patients, the urine examinations were negative, likewise the serological tests.

TABLE I. *Permeability of the Synovial Membrane to P.S.P.*

No.	Diagnosis	Date	Amt. cc. withdrawn	Bact'y	P.S.P. app.	P.S.P. 1 hr.	P.S.P. 2 hrs.	Total
					min.	%	%	%
917	Chronic Multiple Arthritis	12/ 6/'27	120	*Strep. viridans*	9	40	22	62
4745	Osteocartilage Loose Body	6/ 6/'28	50	Neg.	12½	25	30	55
4745	Osteocartilage Loose Body	7/11/'28	90	Neg.	12½	27	20	47
8930	Injured Cartilage	12/31/'28	110	Neg.	9	20	15	35
11124	Traumatic Arthritis	4/16/'29	100	Neg.	12	30	26	56
16808	Injured Cartilage	11/ 5/'29	35	Neg.	13½	25	25	50
				Average	11½	28	23	51

As a means of comparison, the permeability of other serous and synovial membranes was similarly studied in 6 other patients. The *tunica vaginalis testis*, the peritoneum, the synovial lining of the

TABLE II. *Permeability of Serous and Synovial Membranes to P.S.P.*

No.	Membrane	Diagnosis	Date	P.S.P. app.	P.S.P. 1 hr.	P.S.P. 2 hrs.	Remarks
980	Tunica Vag. Testis	Hydrocele	12/ 7/'27	10 hrs.(?)	None	—	85% recovered in 16 hrs.
2621	Peritoneum	Ascites of Portal Cirrhosis	3/ 1/'28	2nd hr.	None	3%	5% 3rd hr. 5% 4th hr.
8255	Tunica Vag. Testis	Recurrent Hydrocele	12/ 4/'28	12 hrs.(?)	None	None	Nearly all recovered in 16 hrs. 400 cells per cu. mm.
10287	Synovial Bursa	Prepatellar Bursitis	10/ 7/'29	(?)	None	None	Numerous cells
11064	Synovial Tendon Sheath	Ganglion	4/29/'29	15 min.	40	20	8% excreted first 15 minutes
9869	Pleura	Carcinoma of Breast Hydrothorax	1/14/'30	2nd hr.	None	5%	15% in 9 hrs.

prepatellar bursa, the synovial sheath of one of the dorsal carpal tendons, and the pleura were thus investigated. The results are presented in Table II. In the instance in which there was a possibility of infection, in the case of prepatellar bursitis, the fluid was sterile bacteriologically. The 5 membranes were extraordinarily variable in their permeability. In the case of the tendon sheath the process of absorption was rapid; the P.S.P. appeared in the urine within 15 minutes and 60% was excreted in 2 hours. In the case of the *tunica vaginalis testis*, however, the process was very slow. In fact, little if any of the dye was excreted by the kidney in 12 hours, and nearly all the P.S.P. injected was recovered from the hydrocele sac at the end of 16 hours. This latter observation is being thoroughly studied by Huggins and Entz.[4]

In traumatic arthritis with effusion the synovial membrane of the knee is freely permeable to phenolsulphonephthalein. There is great variability in the permeability of diseased serous and synovial membranes.

4762

Creatine in Medullated Nerve.

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From the Department of Physiology, University of Chicago.

The finding in frog and rabbit sciatic nerves of a soluble phosphorus compound behaving like the phosphocreatine of muscle[1] suggested the desirability of a similar study of nerve creatine.

Total creatine was determined, after digestion of the whole tissue for 3 hours in N H_2SO_4 on a water bath, by the usual picric acid method. For estimating "free" and "bound" creatine, the cold tissue was extracted with iced trichloracetic, alcohol added to an aliquot portion to a concentration of 66% and the bound creatine precipitated with crystalline $Ba(OH)_2$ (Eggleston,[2] for separating phosphocreatine). The 2 fractions were then heated with acid and determined as above. Controls showed full recovery of creatine in the "free" fraction and phosphocreatine (kindly supplied by Dr. Fiske) largely in the "bound" fraction.

[4] Huggins, C. B., and Entz, F. H., personal communication.

[1] Gerard, R. W., and Wallen, J., *Am. J. Physiol.*, 1929, lxxxix, 108.

[2] Eggleston, P., personal communication.

The total creatine content of green frog sciatics varies markedly with the season or condition of the animals, although values for nerves from one frog agree within 6%. From June 4th to July 27th the values rose entirely regularly in 11 successive experiments a few days apart, from 104 mgm. % to 234 mgm. %. The muscles showed a similar but less regular rise from 440 mgm. % to 570 mgm. % (average 500 mgm. % for 13 experiments). The average total creatine in the nerves, in 30 experiments extending from May to January, was 164 mgm. %. A similar average for the bull frog sciatic, based on 20 experiments, was 135 mgm. %, but in this series fewer of the analyses were made during the periods of high concentration than in the case of the green frogs. Two determinations of total creatine in dog's sciatic yielded 139 and 140 mgm. %.

The sum of the free and bound creatine in a trichloracetic acid extract of nerve or muscle was always less than the total determined directly. The tissue residue from the extraction, when digested with acid, yielded a test for creatine and when this amount was added to that in the extract, the sum equalled the total as directly estimated. In 13 experiments on nerve the direct measurement gave 133 mgm. % total creatine and the sum of the fractions also 133 mgm. %. For muscle, 8 experiments gave: total direct, 498 mgm. %; sum, 490 mgm. %. This affords a further check on the methods. The creatine not extracted from the tissue averaged 35 mgm. % for nerve (36 experiments) and the same for muscle (39 experiments). This could not be removed by repeated extraction with trichloracetic acid, water, mineral acid, alkali, or ether-alcohol, and was not lessened when the tissue was powdered in liquid air before extraction.

The preformed creatinine in nerve was hardly more than in a blank test.

In fresh nerve the free creatine averaged 57 mgm. %, the bound 44 mgm. % (6 experiments). A somewhat larger fraction was bound when the nerves had been allowed to rest in air or oxygen. It is striking that in both nerve and muscle, despite marked differences in the total, about half the creatine is bound. After 8 to 24 hours in nitrogen at 20° C. the values for nerve were 80 mgm. % free, 25 mgm. % bound (7 experiments); about half the bound creatine having been freed. For muscle, the bound creatine fell from 260 mgm. % to 65, over three-fourths being broken down. In CO_2, two experiments have shown a similar though lesser decomposition. The effect of activity is being studied.

Fresh frog nerve contains about 9.5 mgm. % of phosphorus in a

labile combination, of which 4 mgm. % is broken down during asphyxia. If phosphocreatine is the substance there should be 40 mgm. % of bound creatine in fresh nerve, 23 mgm. % after asphyxiation. The observed values are 44 and 25, which leaves little doubt that the labile phosphorus and bound creatine are present in nerve as phosphocreatine. Similar agreement is obtained for muscle.

4763

Effects of Ligating the Bile Duct in the Rat.

FREDERIC T. JUNG.

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

The experiment to be described was suggested by a finding reported by Buchbinder and Kern,[1] namely, that ligation of the common bile duct in the pup is followed by a decline in the blood calcium and by the development of an osteoporotic condition. Since the incisors of the rat are teeth of continuous eruption and relatively convenient for microscopic study, this animal could be expected to show the effects of an icteric calcium metabolism upon growing teeth.

In a series of rats, all of them males, white, weighed every day, and kept on a standard diet, ligation of the bile duct was done with asepsis and ether-magnesium sulphate anesthesia. The duct was found in the hepatoduodenal ligament and was divided between liga-

TABLE I.
Survival-times of Male Rats, Untreated, After Ligation of Bile Ducts.

			Weight	Survival	Remarks
			gm.	days	
1	RH	1	169	2.5	
2	RF	4	147	2.8	
3	LH	2	158	4	
4	LH	45	145	7	
5	RH	3	161	10	
6	LH	4	173	10	
7	LH	1	155	12	Smallest cyst.
8	LH	3B	185	14	Small cyst.
9	LH	34	172	23	Large cyst.
10	LH	5	132	30	Large cyst; ascites.
11	LH	3A	151	38	Large cyst; bleeding from nose, etc.

[1] Buchbinder, W. C., and Kern, R., PROC. SOC. EXP. BIOL. AND MED., 1927-8, XXV, 104.

tures. The survival-times of a series of 13 such animals are given in Table I.

The urine became bright yellow less than 12 hours after the operation and remained so, leaving yellow stains on the hair about the penis. The otherwise pink ears assumed a yellow tint. The feces became lighter in color. Ascites developed in only one rat; spontaneous hemorrhages were observed in one other.

Construction of weight curves showed that it was impossible to tell, from daily observations of the animal's weight, when death was imminent. The operation was always followed by a decline in weight lasting from one to 5 days. In some cases a period of recovery followed, with a normal daily increase in weight. In one striking case death occurred suddenly on the 14th day while the rat was gaining consistently and weighing 14 gm. more than at operation. There was no ascites in this rat. Comparison of weights at operation with survival-times reveals no correlation; this may be partly due to the narrow range of weights in the series studied (132 to 185 gm.).

The rat has no gall-bladder. The distension of the bile-duct proximal to the ligature slowly gives rise to a small sack which, in the beginning, contains bile. Rats autopsied 3 or 4 days after the ligation showed only a filling of the hepatic ducts with a clear yellow fluid. The sack increases continually in size. Rats autopsied after the 12th day showed no more of the clear yellow fluid; the contents of the sack were now colorless fluid containing black flaky material in suspension.

No gross changes were found in the teeth even of the rat which lived 38 days after the ligation. It seemed desirable, therefore, to see whether the combined depressing effects of icterus and thyropara-

TABLE II.

Survival of Rats After Thyroparathyroidectomy and Ligation of Bile Duct.

			Survival-time		Remarks
			After ligation	After thyroparathyroidectomy	
1		3	13 hours	13 hours	Severe convulsions.
2	LF	3	25 "	25 "	Mild tetany.
3	LH	1	3 days	3 days	Very severe tetany.
4	RH	12	4 "	12 "	Very severe tetany before ligation.
5	LF	4	6 "	6 "	Very severe tetany.
6	RH	13	32 "	40 "	Mild tetany before ligation.
7	LH	3C	64 "	64 "	Moderate tetany. The icterus cleared up in about a month, however.

thyroidectomy upon blood calcium would produce any changes.

Five rats were successfully ligated and thyroparathyroidectomized each at a single sitting. Two other rats which had survived thyroparathyroidectomy and had exhibited tetany had their bile ducts ligated at a second operation with the production of jaundice.

None of these rats showed gross dental defects. The microscopic findings will be reported in another paper (Skillen and Jung).

4764

Auto-Disinfecting Power of the Human Skin.

CHARLOTTE SINGER AND LLOYD ARNOLD.

From the Research Laboratory, State Department of Public Health, and University of Illinois, College of Medicine, Department of Bacteriology and Preventive Medicine, Chicago.

The skin was cleaned with soap and warm water. Broth cultures of *B. prodigiosus, B. pyocyaneus, B. coli, B. typhosus, B. paratyphosus, B. enteritides, Staphylococcus aureus* and *albus* have been used as test micro-organisms. Roughly 90 to 95% of the number of bacteria applied to clean skin are rendered non-viable within 10 minutes. The coli-typhoid group are destroyed more rapidly than other bacteria. The endogenous *Staphylococcus epidermis albus* is removed slower than the air strains of the *Staphylococcus albus.*

When dirty or fatty skin is exposed to the same bacteria under similar conditions they are destroyed slowly, depending among other things, upon the thickness and impermeability of foreign fatty layer covering the skin. Viable bacteria can be determined several hours after being applied to such fat covered skin.

The finger-nail region is an exception to the self-disinfecting power of other skin area. The region under the finger-nail at the tips of the fingers always contains a large number of *Staphylococcus epidermis albus.* Test bacteria applied to this region remain viable for longer periods of time.

4765

Classification of Staphylococcus Epidermis Albus From Human Skin.

B. MONTGOMERY AND C. J. GUSTAFSON. (Introduced by Lloyd Arnold.)

From the Department of Bacteriology and Preventive Medicine, University of Illinois College of Medicine, and Research Department of the State Department of Public Health, Chicago.

Staphylococci were obtained from the skin (palmer and dorsal surfaces of hand, over chest and back), under the nails at the tips of fingers and from the margins of the nail on their dorsal surface. Sterile swabs moistened with saline were used to obtain specimen. Standard technical procedures and media were used. Seventy-five strains from the skin, under the nail and the dorsal margin of the nails were classified. The production of acid upon lactose, maltose, dextrose, mannite and litmus milk and the reduction of nitrates and the liquefaction of gelatin were determined. Acid production upon lactose media proved to be the differentiating reaction. Eighty-four per cent of the skin strains of *Staphylococcus epidermis albus* fermented lactose, half of the same strains of staphylococci from the dorsal margin of the finger-nail fermented lactose and half did not produce acid from this sugar. Only 15% of the *Staphylococcus epidermis albus* isolated from under the nail at the tips of the fingers fermented lactose, 85% did not produce acid when grown in contact with this sugar.

All of the strains are practically non-pathogenic for mice, conforming to the usual findings in this respect. The work is being continued to ascertain if post-operative and other pyogenic infections contain the skin or under the nail strains of *Staphylococcus epidermis albus.*

4766

Gastro-Intestinal Port of Entry of B. Tuberculosis in Guinea Pigs.

C. GUSTAFSON AND LLOYD ARNOLD.

From the Research Laboratory, State Department of Public Health, and University of Illinois, College of Medicine, Department of Bacteriology and Preventive Medicine, Chicago.

We have been able to demonstrate differences in the permeability of the wall of the small intestine by changing the contents of the lumen. Certain bacteria injected into the duodenum could be found in a viable condition in the thoracic duct lymph.[1] Fresh egg white, 1 to 2% solutions of desiccated ox bile and alkaline phosphates seemed to influence intestinal permeability, fresh egg white producing the maximum effect. Saline suspensions of bacteria injected into the duodenum in the same manner could not be demonstrated in the thoracic duct lymph. The test bacteria could be demonstrated in the mesenteric lymph glands in the dogs in all instances after intraduodenal injection. All experiments were performed under local anesthetic. We wished to test the permeability of the intestinal wall in guinea pigs to *B. tuberculosis* as a result of the above mentioned observations upon dogs. We used intraduodenal injections to rule out some of the factors associated with oral administration and the subsequent uncontrollable gastric environment.

Under ether anesthetic the abdomen of guinea pigs was opened, the duodenum exposed and 5 cc. fluid was injected. Saline, fresh egg white, 2% desiccated ox bile, 1/15 M. disodium phosphate and an equal mixture of egg white and bile were the fluids used. As control, 6 guinea pigs were injected with each of these 5 solutions. The animals remained healthy for the 3 months' period of observation. Bovine strains of *B. tuberculosis* were used, 5 mgm. was suspended in 5 cc. of each of the above mentioned solutions and injected intra-duodenally. Thirty guinea pigs received the saline, 19 egg white, 17 bile, 17 alkaline phosphate and 17 egg white and bile, a total of 100 guinea pigs. We have eliminated all accidental deaths from this series. All guinea pigs after 6 weeks' observations were killed, 100% showed tuberculosis. The majority showed extensive generalized tuberculosis.

The guinea pig is so susceptible to the *B. tuberculosis* that all of the suspensions proved effective in producing tuberculosis following intra-duodenal administration.

[1] Arnold, *J. Hyg.*, 1929, xxix, 82.

4767

Gastro-Intestinal Port of Entry of B. Tuberculosis in Dogs.

JANET THAYER, C. GUSTAFSON AND LLOYD ARNOLD.

From the Department of Bacteriology and Preventive Medicine, College of Medicine, University of Illinois, and Research Laboratory, State Department of Public Health, Chicago.

Arnold[1] reviewed the work done in this laboratory upon the permeability of the intestinal tract to bacteria as a result of certain environmental changes. This is a report of our attempt to utilize similar experimental procedures to determine the permeability of the intestinal tract of the dog for *B. tuberculosis*.

Young dogs (2 to 4 months old) were used for these experiments. Some animals were given doses of *B. tuberculosis* in milk and placed upon a general bread and milk diet for 4 weeks. Others were fed a dose of *B. tuberculosis* and killed between 1 and 2 hours after feeding. A third experiment consisted of injecting the *B. tuberculosis* directly into the lumen of the duodenum and examining certain tissues 30 minutes later. The mesenteric lymph gland was removed from all dogs immediately after killing them in the ether chamber. The gland was macerated by grinding in mortar with sand and 3% sodium hydroxide, neutralized with hydrochloric acid and injected into 2 guinea pigs.

Forty-five young dogs were fed 3 times, once daily for 3 days, 10 mgm. *B. tuberculosis* in 100 cc. milk. Fifteen animals were given plain milk (pH 6.7) 8 of these were in ordinary temperature rooms, 7 were in warm and humid rooms (90° F. and 90% relative humidity); the same experiment feeding lactic acid milk (pH 5.0) and feeding alkaline milk (pH 8.0). These animals were observed for 6 weeks. Six died during this time, all had *B. tuberculosis* in mesenteric lymph gland by guinea pig test. These 6 puppies were undernourished and in poor condition at the beginning of the experiment. Only 3 of the remaining 39 animals showed positive *B. tuberculosis* by guinea pig tests. There was no evidence of gross lesions in any of these 9 animals. There was no difference between hot and ordinary room environment or in the acid, alkaline or plain milk animals.

The next experiment consisted of giving 62 puppies, one feeding each of 100 mgm. of *B. tuberculosis* in various milks, and removing mesenteric lymph glands one hour after feeding. Plain milk, lactic

[1] Arnold, *J. Hyg.*, 1929, xxix, 82.

acid milk and alkaline milk was fed to 36 animals, 12 each, in ordinary temperature room. In the warm and humid room, 16 were fed plain milk, 8 each with lactic acid and alkaline milk. The mesenteric lymph glands for all 62 young dogs were negative by the guinea pig test.

Under ether anesthesia 50 cc. of saline containing 1 gm. desiccated ox bile, one fresh egg white and 100 mgm. *B. tuberculosis* was injected into the lumen of the duodenum of 7 dogs. 10 cc. of portal vein blood was removed 5, 10 and 15 minutes after intra-duodenal injection. Animals were killed 30 minutes after injection. The portal blood specimen and parts of liver showed an absence of *B. tuberculosis* by the guinea pig test.

4768

Antigenic Absorption Through Intact Vaginal Mucosa in Humans.

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From the Departments of Bacteriology, Preventive Medicine and Obstetrics, and Gynecology, University of Illinois, College of Medicine, Chicago, Ill.

The introduction of an antigen into the body by applying it upon some surface area has received considerable attention since Besredka's work upon this subject. We wish to report the results obtained so far upon the application of an antigenic substance upon the vaginal mucosa and the demonstrable antibody titer in the blood serum following the contact of the antigen with this body surface. The application of the antigenic substance upon the intestinal mucosa in the light of our previous observations is now being attempted.[1, 2]

B. typhosus broth cultures were lysed by phage and passed through the Berkefeld filter. The vaginal mucosa was exposed with aid of a speculum and 1 cc. of the sterile *B. typhosus* proteins sprayed upon the wall of the vagina. Blood was obtained before and each week for 3 weeks after the one application of 1 cc. of the antigen.

Ninety-eight cases have been followed through the period of observation. Of these, 75 or 76.5% showed the presence of agglutinins after 3 weeks, 23 or 23.5% were negative. Table I shows

[1] Arnold and Finder, PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 615.

[2] Arnold, *J. Hyg.*, 1929, xxix, 82.

TABLE I.
B. typhosus proteins (dissolved by phage) applied to mucosa of vagina. Agglutination titer 3 weeks after application.

No. Cases	Dilution	%
9	1:20	12.0
36	1:40	48.0
29	1:80	38.7
1	1:160	1.3

the average titer of agglutinins in the 75 positive cases. A group of 40 of the 98 cases were tested each week for 3 weeks. After 7 days 63% showed positive agglutination, 14 days, 72%, and after 21 days 80%. The agglutination titer remains constant for at least 6 weeks. Further work is in progress upon the antigenic absorption in pregnant women. The work upon the oral administration of antigens has not been completed.

4769

The Electrical Resistance of Live and Dead Tissue.

A. BACHEM.

From the College of Medicine, University of Illinois, Chicago.

It can be shown that the stationary temperature which results after sending an electric current through tissue for a considerable time depends mainly on 4 factors: (1) the current intensity, (2) the cross section of the tissue penetrated by the current, (3) the specific resistance of the tissue, (4) and the heat conductivity of the tissue, which depends primarily upon the blood circulation. The importance of this factor has been demonstrated conclusively by a recent investigation about the effect of electric currents upon the blood vessels.[1]

The specific resistance is of prime importance, because, in addition to determining the heating effect of a given current, it also influences the path of the current, as soon as this resistance varies over the volume of tissue in question. Therefore the experimental determination of the resistance of the various tissues of the human body as of the animal is of great importance.

Omitting the meagre literature[2, 3] about the subject I will describe

[1] Jaffé, Willis, Bachem, *Arch. Path.*, 1929, v, 244.

[2] Wildermuth, *Mittlgn. a. d. Grenzgeb. d. Med. u. Chirurg.*, 1911, xxii, 511.

[3] Dowse and Iredell, *Arch. Radiol. a. electrother*, 1920, 33.

shortly the experiments which I have conducted partly in collaboration with W. S. Brown:

(1) Eight different organs of a dog were exposed to 3 kinds of current: a. Direct current; b. an alternating current; c. a high frequency current.

(2) These experiments were made under 3 different conditions: (a) *in vivo*, the dog being anesthetized; (b) the organs being left in position, right after the dog had been killed; (c) the organs removed, kept on ice for one night and then brought back nearly to room temperature.

(3) The same organs were taken from fresh bodies and exposed to the same 3 kinds of currents.

By these experiments could be determined: (1) the individual resistance of each organ; (2) the effects which are due to the special character of the current; (3) the influence of the condition of the material (live, dead, fresh, old, etc.); special care was taken in case of the lungs (inflated, collapsed).

The figures obtained from these measurements indicate that the resistance depends very strongly upon the current used. It is smallest for high frequency, medium for low frequency and greatest for direct current. In the latter case the resistance is by no means constant; it increased rapidly in the beginning and slowly later on.

Another result obtained is the change from live to dead tissue. In most cases there is a tendency toward an increase of resistance from life to death; the resistance is still higher after the tissue is kept on ice for a day. An exception is the skin; the resistance decreases markedly from life to death.

A particularly pronounced resistance change was found for the lungs, while inflated and after collapsed. The first observation was made on the extended lungs, with the trachea ligated; then the lungs were allowed to collapse and a second reading taken while the animal was still alive, the third reading was made right after the animal died. The lungs were removed, kept on ice, and a fourth reading made a day later. The drop of resistance is most pronounced at the moment of collapse; from then on there is still a noticeable decrease.

The figures obtained for the human body correspond to the last ones for the dog. In order to get an estimate for the live human tissues one would have to decrease the resistance in the ratio as found in the dog experiment; for the skin, and particularly for the inflated lungs the figures would have to be increased correspondingly.

A few outstanding facts from these observations are the compara-

tively low resistance of skin for high frequency as compared to the immense resistance toward DC; and the fact also that no difference occurs in case of HF for dry and moistened skin. The resistance of bone depends very pronouncedly upon the specimens observed; it was comparatively small for the porous bone of the skull (calvarium); it was very high for the solid bone of the tibia.

Conclusions: (1) The study of electrical resistance should be made with live material because pronounced changes occur in dead tissue. (2) The tissues vary so much as to resistance that the course of the electric current is affected and a great variation of heat production should be expected. This is important for the placing of electrodes. (3) Skin resistance is much greater than the resistance of the average tissue for DC; it is a little greater for HF. Therefore direct current seems more useful for skin cautery while high frequency is less irritative to the skin and more useful for deep therapy.

4770

The Electrocardiogram in Experimental Obstructive Jaundice.*

WILLIAM C. BUCHBINDER.

From the Cardiographic Laboratory of the Michael Reese Hospital and the Nelson Morris Institute for Medical Research, Chicago.

In a previous communication[1] it was noted that there is an age factor in the production of bradycardia in dogs following ligation and division of common bile duct. A slowing of the heart action was observed only in the puppy; in the adult animal a slight acceleration of the heart rate was the rule. The heart rate was determined by ausculation supplemented, in the puppy, by the electrocardiogram. The present investigation purposed to note whether the heart rate of the adult animal as determined by the latter method was in agreement with ausculatory findings, and also to elucidate electrocardiographic abnormalities.

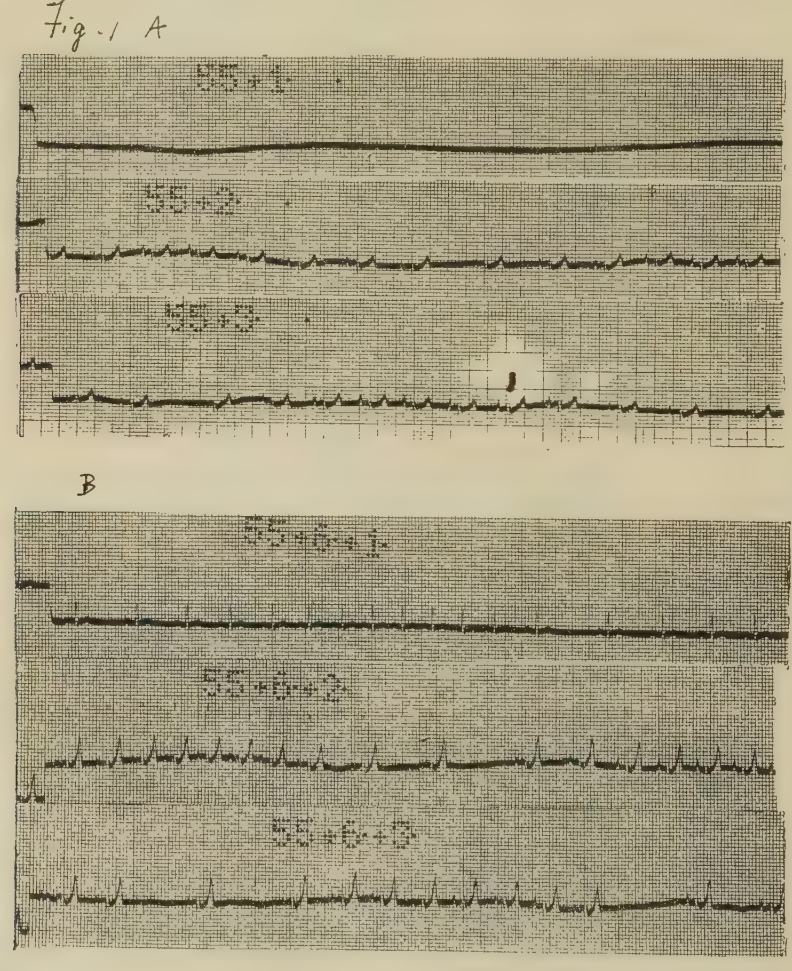
This report is based upon a study of the curves obtained from 13 of 21 animals subjected to ligation and division of the common bile duct. Eight animals revealed extensive biliary or other infection at autopsy; the data that these furnished were derived from the control

* Aided by the Emil and Fanny Wedeles Fund for the Study of Diseases of the Heart and Circulation, and in part by a grant from the Douglas Smith Foundation of the University of Chicago.

[1] Buchbinder, William C., *Arch. Int. Med.*, 1928, xlii, 743.

curves. Electrocardiograms were made at 3 to 4 day intervals before and after the induction of jaundice, the animal lying quietly on its right side.

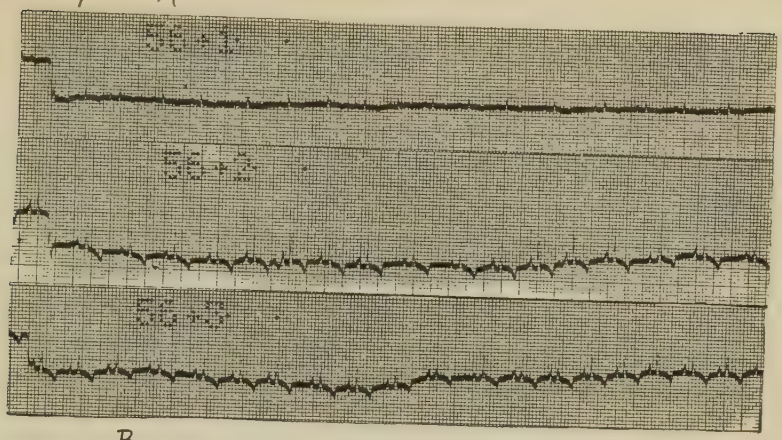
The essential change noted in the electrocardiogram following ligation and division of the common bile duct was an accentuation of the breathing arrythmia, normally present in a dog. This was seen in the curves of 9 of the 13 animals (Figs. 1B and 2B). A slight in-



A. Control curve. Rate approximately 105. B. 8 days after ligation and division of the common bile duct. There is a marked accentuation of sinus arrythmia. The increase of the R-R interval might conceivably be interpreted as being due to sinus block. Rate approximately 115. There is an increase in the voltage of R and T, especially the latter.

NOTE: In these and the following records the ordinates are 0.04 of a second apart; the abscissae 10^{-4} volts. Standardization was such that 1 cm. equals 1 millivolt.

Fig. 2 A



B

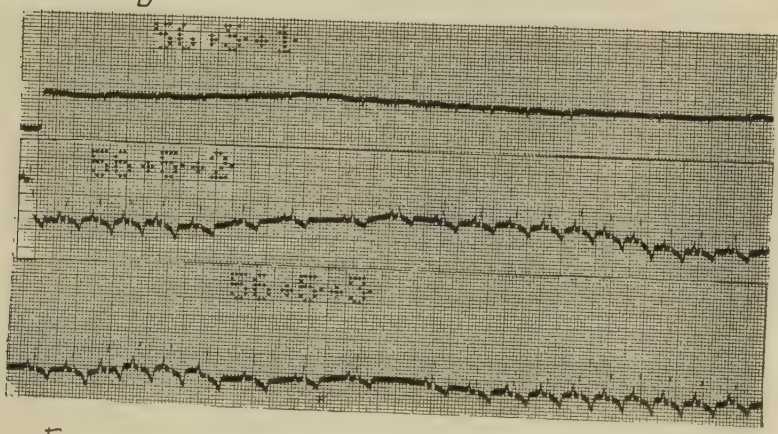
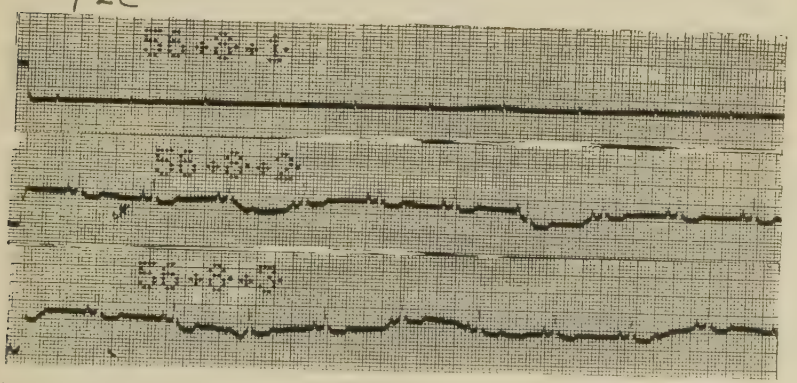


Fig. 2C



A. Control curve. Rate approximately 125. There is some slurring of the QRS group. B. 5 days after ligation and division of the common bile duct. Marked accentuation of sinus arrythmia. Rate approximately 135. P and R are inverted in the first lead as the result of a slight shift of the anatomic axis of the heart. C. 15 days after ligation and division of the common bile duct. Animal moribund. Sinus slowing. Rate 75. There is a marked splitting of the P and R waves.

crease in the heart rate usually occurred during the inspiratory phase; at the end of expiration there was a prolongation, relative or absolute, of the R-R interval. The two phases of respiration therefore became more sharply demarcated from one another. As a result the heart tended to be slightly more rapid than before the induction of jaundice, thus confirming the findings obtained by auscultation. A more marked tachycardia occurred in the curves of 4 animals, sinus arrythmia here almost disappearing. A marked sinus bradycardia was seen in the electrocardiogram of only 1 animal, but only after it became moribund.

Excluding the records of several animals in this state or at the point of exitus (Fig. 2-C), the electrocardiogram showed no significant departure from the normal after the induction of jaundice. There was no increase in the conduction time of the heart. The voltage of R and T remained unchanged except in 2 curves late in obstruction when they were concomitantly increased. Slight changes in the amplitude of R in the first and third leads frequently seen in the dog, purely respiratory effects, became a little more conspicuous with the accentuated sinus arrythmia. An extrasystolic arrythmia was not seen in any of the electrocardiograms. Inversion of the p wave was encountered twice; it was transient and in one instance could be accounted for by a shift of the anatomic axis of the heart, due, probably to fluid accumulation into the peritoneum.

A directional change of the T wave after the induction of jaundice could not be determined after a study of the curves. Since this wave is notably unstable in the dog the evaluation of any morbid process as affecting a change in its direction becomes increasingly difficult. Thus the control electrocardiograms of only 7 of the 21 animals showed T waves having a uniform direction, those of 2 displaying always an upright, and of 4 always a downward deflection in all leads. The incidence of negative and positive deflections from a total of 63 control curves derived from the 21 animals was as follows: In the first lead there were 24 positive and an equal number of negative deflections. In the second and third leads 23 negative as opposed to 28 positive deflections. Sixteen consecutive tracings of one animal subjected to a mock operation after the usual 3 were obtained, revealed a similar lack of uniformity in the direction of the T wave. This inherent instability of the T wave would have manifested itself, in all probability, in all instances had sufficient curves been made of those in which it appeared stable. From these data it would be hazardous to conclude that jaundice influences its direction. On the other hand a parallelism in the change of its direc-

tion in the second and third leads throughout the period of obstruction is strong argument against any directional change of the T wave in jaundice.

Summary: An accentuation of sinus arrythmia constitutes the essential change in the electrocardiogram of adult dogs subjected to ligation and division of the common bile duct. A slight increase in the heart rate occurs in the jaundiced adult dog. Obstructive jaundice in this animal does not appear to produce any directional change of the T wave.

New York Section.

New York Academy of Medicine, February 19, 1930.

4771

Bacteriophage in Relation to Healing of Osteomyelitis.

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(Introduced by W. J. MacNeal.)

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In the treatment of osteomyelitis by the method of Orr, the open wound is protected by vaseline gauze under slight pressure and immobilized by plaster dressings. It is then left undisturbed for long periods. Success evidently depends in large measure upon the effective control of infection by the humors and cells of the patient. The possible influence of bacteriophage requires consideration.[1]

A boy of 10 years, presenting open sinuses leading into the right tibia, the left tibia and the left humerus resulting from an original haematogenous osteomyelitis twice subjected to surgical operation elsewhere, and of one year's duration, was again subjected to surgical operation for removal of sequestra from both legs on September 9, 1929.

Pus from the right tibia was examined bacteriologically at this time, again at the first subsequent dressing on November 11, on December 9 and on December 23. Bacteriological examination of material from the left tibia was made on November 11 and December 9 and of material from the left humerus on November 7, December 9 and December 23. Native bacteriophage was not found in the arm wound. A stock anti-staphylococcus bacteriophage lytic for the wound strain, was introduced into the wound of the arm on December 9, which was followed by complete closure of the wound in 2 weeks. The data of these examinations are summarized in Table I.

[1] Albee, F. H., *Int. J. of Med. and Surg.*, 1929, xlii, 1.

TABLE I.—*Chronic Multiple Osteomyelitis.*

Right Tibia	Left Tibia	Left Humerus
	(Organisms are listed in order of their predominance)	
First specimen, Sept. 9. Hemol. *Staph. aureus* *B. coli* and active native anti-coli phage		
Second specimen, Nov. 11. *B. coli* *B. pyocyaneus* Native anti-coli phage	First specimen, Nov. 11. Non-hemol. *Staph. aureus* Hemol. *Staph. aureus* *B. coli, B. pyocyaneus* Native anti-coli phage	First specimen, Nov. 7. Hemol. *Staph. aureus* Phage not detected
Third specimen, Dec. 9 Diphtheroids *B. coli* *B. pyocyaneus* Phage not detected	Second specimen, Dec. 9 *B. pyocyaneus* Diphtheroids Phage not detected	Second specimen, Dec. 9 Diphtheroids Hemol. *Staph. aureus* Stock anti-staphylococcus phage introduced
Fourth specimen, Dec. 23 *B. coli* *B. pyocyaneus* Hemol. *Staph. aureus* Phage not detected Wound ¼ area of Sept 9.	Dec. 23 Wound healed	Third specimen, Dec. 23 Diphtheroids Hemol. *Staph. aureus* Anti-staph. phage recovered from scrapings Wound healed

The bacteriophage in the first specimen of pus from the right tibia was at once active against 2 stock strains of *B. coli.* Of greater interest is the fact that it was also active against the native strain of *B. coli* in the pus, so that after 5 serial filtrations of cultures of this organism, complete lysis was obtained with a titer of 1×10^{-8}.

A woman aged 21 was admitted with a compound comminuted fracture of left radius and ulna due to automobile accident 2 months before. Bone fragments were now presenting through the sloughing wounds. Sequestrectomy and dressing by the Orr technic was carried out. The pus contained *B. coli* (acidi lactici) and lesser numbers of *Staphylococcus aureus,* both susceptible to lysis by stock bacteriophage strains in the laboratory collection. The pus also yielded a native bacteriophage, ineffective against the bacteria of the wound but active against a laboratory strain of staphylococcus, by means of which its lytic power was exalted so that it eventually produced complete lysis of the wound strain of staphylococcus. At the first dressing, 8 weeks after operation, the same bacteria were recovered from the wound, the staphylococcus now predominating. Bacteriophage could not be found at this time although the staphylococcus strain from the wound was still lysed by the regenerated and exalted native bacteriophage obtained at the first examination. The wound presented a satisfactory appearance. Seven weeks later,

examination of the exudate yielded only *Staphylococcus aureus*. The opening on the dorsal aspect had healed and the volar opening was in very satisfactory condition.

A man of 40 was admitted with osteomyelitis of left tibia of 36 years' duration. The sinus was widely excised and an Orr dressing applied. Pus taken at this time showed predominating hemolytic *Staphylococcus aureus* and a few diphtheroids. The pus also yielded a bacteriophage active against a stock strain of staphylococcus and, after exaltation, lytic for the wound strain. At subsequent dressings the diphtheroids predominated and only a few colonies of staphylococcus were obtained. The bacteriophage could no longer be detected. Satisfactory healing was obtained after approximately 4 months.

These preliminary observations have convinced us that neither the bacteriologist nor the surgeon should ignore the significance of bacteriophage in infected wounds, particularly those involving bone.

4772

The Electric Charge of Mosaic Virus Particles.

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Recently a number of ultramicroscopic viruses of man and animals, and bacteriophage have been found, in general, to migrate to the anode under ordinary conditions of hydrogen ion concentration.

In the controlled experiments to be reported, a study was made of a typical virus of plants, namely that of mosaic disease in the tomato with the object of noting (a) the possible migration of a plant virus in an electrical field, (b) the direction of migration, and (c) any difference of behavior of unfiltered and filtered suspensions for a considerable amount of protein particles are removed from the latter.

The method of cataphoresis employed has already been described.[1] Tests were made at 4 m. a., 118-119 volts P.D., over a period of 3 hours. Suspensions of ground mosaic-infected leaves were prepared in G.P.A.,[2] or in phosphate buffer solutions at pH=5.3 to 8.5, with

[1] Olitsky, P. K., and Long, P. H., *J. Exp. Med.*, 1929, i, 263.

[2] Northrop, J. H., and de Kruif, P. H., *J. Gen. Physiol.*, 1922, iv, 639.

final dilutions of 1:300 to 500. Filtrates were obtained by single filtration through Berkefeld "N" candles. The anodic and cathodic materials were inoculated respectively into each of 5 to 10 normal tomato plants. The results are tabulated as follows:

TABLE I.

A. Unfiltered Material

pH	5.3		7.5		8.2	
Pole	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Mosaic (+) or	5+	0+	5+	0+	5+	0+
Unaffected (—)	0—	5—	0—	5—	0—	5—
Average incubation period (days)	13.4		12.6		11.4	

B. Filtered Material

pH	5.3		7.0		7.5		8.2		8.5	
Pole	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Mosaic (+) or	6+	0+	4+	0+	1+	0+	2+	0+	3+	0+
Unaffected (—)	4—	10—	1—	5—	4—	5—	3—	5—	2—	5—
Average incubation period (days)	13.5		10.5		12		14.5		16	

It would appear, therefore, that mosaic virus or particles containing the virus migrate to the anode in an electrical field, at pH readings of 5.3 to 8.5. Thus this plant virus agrees in this respect with most viruses of mammalian origin and with bacteriophage. Filtration does not interfere with this property; the qualitative results are as clear cut as with unfiltered suspensions; the quantitative differences are probably due to the well-known factor of diminution of infective power after filtration of the mosaic virus. In a control test of filtrate inoculations, only 3 of 10 plants showed mosaic disease after about 11 days. It was also noted that a greenish clouding, resulting from protein and chlorophyll deposits, occurred only at the anode in both filtered and unfiltered suspensions, at hydrogen ion concentrations of from 7 to 8.5.

4773

Effect of Cysteine on the Survival of Vaccine Virus.

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It is well known that ultramicroscopic viruses rapidly lose their power to infect when kept at 37° C., and become wholly inactive in a few days. If the viruses, however, are placed under anaerobic conditions, they maintain activity for a longer period of time.

The following experiments were made with neurovaccine virus for the purpose of noting the effects of an active reducing agent, cysteine hydrochloride.

The virus was derived from infected rabbits' testicles and suspensions of the tissue were prepared in Ringer's solution, distilled water, and veal-infusion broth, the final dilutions being 1:50. Twenty tubes each containing 10 cc. of the respective suspensions were prepared and to each was added sufficient cysteine hydrochloride adjusted to pH = 7.5 to make a 1:2,000 dilution of the chemical. A similar series of tubes without cysteine completed the series. All tubes were sealed with petrolatum and kept at 37° C.

After 7, 14, 21, 32, 42, 54, and 83 days, the material was tested for activity by the inoculation of different dilutions into the shaved skin of rabbits. The effect of the cysteine on the survival of the virus is shown in the table:

TABLE I.

Material plus virus	7 days	14 days	21 days	32 days	42 days	54 days	83 days
Ringer's solution	1:50±	—	—	—	—	—	—
Ringer's solution + cysteine	1:50,000	1:500,000	1:500,000	1:500,000	1:500,000	—	—
Distilled water	1:50?	—	—	—	—	—	—
Distilled water + cysteine	1:50,000	1:500±	1:50,000±	1:500,000	1:50	1:50±	—
Broth	1:50	1:50	—	—	—	—	—
Broth + cysteine	1:5,000	1:50	No record	1:500	1:500	1:5,000±	1:500

± = mild reaction; — = no reaction; ? = doubtful reaction, and the numbers represent the highest dilution in which infectivity was noted. The virus by itself was immediately active in 1:100,000,000 dilution at the time of preparation.

It follows, therefore, that cysteine hydrochloride definitely favors the survival of vaccine virus at 37° C., and the broth vehicle pro-

longs the period of viability to a greater degree than either Ringer's solution or distilled water.

4774

Electrophoretic Mobility Velocities of Rough and Smooth Avian and Bovine Tubercle Bacilli.*

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Falk[1] showed that the electrophoretic mobility velocity (potential difference) of diphtheria bacilli varied according to their virulence, the more virulent organisms showing low mobility rate, while those of a less virulent nature gave a higher reading. For pneumococcus,[2] on the contrary, he found that the potential difference is higher the greater the virulence for white mice and *vice versa*. The sequence of decreasing potentials was shown to be Types III, I, II, IV, which follows the decreasing virulence for white mice. This work on the pneumococcus was carried out prior to the isolation of several new types from the erstwhile group IV.

Following the technique of Petroff[3] the writers undertook the dissociation of a stock strain of avian tubercle bacillus procured originally from Dr. Krumwiede of the Research Laboratories, New York City Department of Health. When the culture was planted on Proskauer and Beck medium after a suitable period of incubation at 37° C. the organism had dissociated into rough and smooth types of colonies. Representatives of each were selected and planted on glycerine agar and on Petroff's egg medium slants. After 5 generations the organisms are still truly representative of the original parent colonies from which they were taken. Other tests were performed on rough and smooth avian and bovine colonies dissociated by Dr. Petroff.

In view of the hypothesis that smoothness of colony may be an indication of virulence, while roughness may be taken as an indication of the reverse, the writers determined the mobility veloci-

* This experiment is part of a group investigation being carried on in conjunction with the Medical Research Board, National Tuberculosis Association.

[1] Falk, I. S., and Jensen, L. B., *J. Bact.*, 1928, xv, 367.

[2] Falk, I. S., *J. Infect. Dis.*, 1925, xxxvii, 481.

[3] Petroff, S. A., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 632.

ties for the two types of colonies, using the Falk slide cell technique. The growths were washed once in distilled water just prior to the experiment and the procedure was adhered to as outlined originally by Falk.

Certain sources of error must be taken into consideration when conducting experiments of this nature: 1. Difference in level of the water table in the cell makes a single standardization of the cell invalid. As it is impossible to get the identical amount of bacterial suspension in the cell, at all times, it is *absolutely essential* that the cell be standardized for the critical zones prior to each and every individual experiment; otherwise, highly variable and inconstant results will be obtained. 2. The lower zone of the slide cell was found to give less variable readings than the upper zone; therefore the results in these experiments were computed with readings taken only from the lower zone. 3. Age of culture examined is a third source of variability; therefore readings should be made on young cultures of identical age and from culture media of identical composition. When these factors are taken into consideration differences in mobility velocity are apparent for the rough and smooth types of colonies. Whether this difference is correlatable with virulence (in the pathogenic sense) as in the diphtheria group must perforce await the outcome of animal inoculations at present under way.

Under the conditions of this experiment the average mobility velocities for the rough avian colonies Krumwiede strain was 20.8 micra per second or 1.74 micra per second per volt per cm. For the smooth Krumwiede strain the average was 37.4 micra per second or 3.11 micra per second per volt per cm. For the Petroff avian strain the average for the rough colonies was 18.4 micra per second or 1.53 micra per second per volt per cm., while for the smooth the average was 35.4 micra per second or 2.95 micra per second per volt per cm. The Petroff bovine strain (B-1) revealed an average reading of 35.9 micra per second or 3.00 micra per second per volt per cm. for the rough colonies, while the smooth colonies revealed an average reading of 50.8 micra per second or 4.23 micra per second per volt per cm. In the case of colony No. 3 bovine smooth there was one reading of 39.0 micra per second. Twenty readings on suspensions from each of 3 other colonies, however, were well within the zone approximated by the average.

Whether these recorded differences in P. D. indicate a difference in virulence remains to be seen. The indications from Petroff's[4] work are that the smooth colony dissociates show a higher virulence

[4] Petroff, S. A., *Am. Rev. Tuberc.*, 1929, xix, 9.

than do the rough. It is interesting, therefore, to note these differences in the electrophoretic mobility velocities of avian and bovine tubercle bacilli from S. and R. colonies.

The E.M.F. used in all of these experiments was 42 volts.

The distance between the electrodes in the cell was 3.5 cm.

4775

Observations on the Pathogenesis of the Myeloid Leucemia of Fowls.*

J. FURTH.

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In a preceding communication[1] a new transmissible strain of leucemia has been briefly described. In the subsequent passages of this strain several of the inoculated birds died of intercurrent diseases chiefly of an acute or subacute inflammatory process of the upper respiratory tract. Birds dying of such infections but not inoculated with leucemic material did not show the pathological changes characteristic for the leucemias. The early death of several birds permitted a study of the pathogenesis of myeloid leucemia. A conspicuous result is obtained when the sequence of events in the development of the organ and blood changes is reconstructed from the following table which includes all the autopsies of 2 recent passages.

An extensive hyperplasia of the bone marrow unaccompanied by a rise in the number of the circulating white blood corpuscles appears to be the first marked pathological change which follows the inoculation of leucemic blood (Stage I). The hyperplasia of the bone marrow consists of an enormous extravascular proliferation of myelocytes and their precursors replacing the fatty tissue and narrowing the blood sinuses. Following this alteration of the bone marrow there is a rise in the number of white corpuscles in the peripheral circulation due to an invasion of cells similar to those found in the bone marrow, but extramedullary blood formation is absent (Stage II). There is a tendency for a further increase of the immature

* This investigation has been supported by a Fund for the Study of Leucemia and Related Diseases.

[1] Furth, J., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 155.

TABLE I.

Number of chicken	Material used for inoculation	Amount injected	Time from inoculation until death	Hyperplasia of bone marrow	Blood changes (white cells)	Extramedullary blood formation
		cc.	days			
467	plasma 361	2.0	47	none	none	none
480		1.0	3	moderate†	”	”
477		0.75	7	doubtful	”	”
478						
476	“white cell layer” 361	0.5	21	extreme	moderate increase	incipient
479		0.25	21	advanced	none	none
475		0.5	23	much advanced	moderate increase	moderate
473		1.0	7	none	none	none
471		1.5	12	slight	”	”
468		1.0	20	much advanced	slight increase	incipient
470	“red cell layer” 361	0.5	23	extreme	moderate increase	none
469		0.5	35	much advanced	none	”
362	blood 266	2.5	36	extreme	enormous increase	moderate
361		2.5	40	”	enormous increase	”
356		2.5	15	slight	none	none
354	organ emulsion 266	2.5	77	extreme	enormous increase	advanced
350	filtrate 266	3.0	70	none	none	none

† The hyperplasia of the bone marrow of this bird was mixed, lymphocytic and myelocytic, whereas in all other cases the hyperplasia was almost exclusively myelocytic and myeloblastic.

The autopsy findings of birds of other passages are similar to those of the above series.

granulocytic elements in the blood stream, a concentration of the latter in the smaller blood vessels (leucostasis) and the development of extramedullary granulocytopoeitic foci (Stage III). The organs most commonly involved are the liver and the spleen. In the liver, the myeloid tissue begins to proliferate in the periportal connective tissue about the adventitia of the vessels extending by extravascular growth. The type of cells found in these foci are similar to those seen in the bone marrow. Mitotic figures are usually numerous in the bone marrow as well as in the extramedullary blood-forming tissues. They are not unfrequent in the circulating blood.

These findings furnish experimental support for the view derived

from human pathology (*Cf.* Askanazy[2]) that leucemia is the sequence of a tumor-like proliferation of the bone marrow which, in a few instances, is the sole site of the pathological process. A mere entrance of immature elements into the blood stream as is the case when leucemic blood injected into the vein of healthy animals does not result in extramedullary blood formation. This appears to take place only after extreme hyperplasia of the bone marrow has been established.

4776

Changes of Blood Gases and Lactic Acid After Exercise in Patients With Rheumatic Heart Disease.

HERBERT W. SCHMITZ AND ELIZABETH SHERMAN.
(Introduced by R. H. Halsey.)

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It has been demonstrated by a number of investigators[1] that changes in the oxygen unsaturation, carbon dioxide combining power, and lactic acid content of the venous blood occur in normal individuals after exercise. These changes seem to bear a definite relation to the amount of work performed. Very slight, or no differences are observed following mild exercise. When the exercise or amount of work performed is increased, greater changes are noted. The changes consist of an increase in the oxygen unsaturation and lactic acid content, and a decrease in the carbon dioxide combining power.

The object of the present study was to determine what changes occurred in the oxygen unsaturation, lactic acid, and carbon dioxide combining power of the venous blood in patients with rheumatic heart disease after performing measured amounts of work. The cases included in this study were children between the ages of twelve and sixteen years, with various valvular and muscular defects caused by the rheumatic infection. Their response to effort was

[2] Askanazy, M., *Handbuch der speziellen pathologischen Anatomie und Histologie*, V. I. pt. 2, Berlin, 1927.

[1] Lundsgaard, C., and Möller, E., *J. Biol. Chem.*, 1923, iv, 315, 477, 599; Barr, D. P., and Himwich, H. E., *J. Biol Chem.*, 1923, iv, 525, 539; Barr, D. P., Himwich, H. E., and Green, R. P., *J. Biol. Chem.*, 1923, iv, 495; Hewlett, A. W., Barnett, G. D., and Lewis, J. K., *J. Clin. Inv.*, 1926, iii, 317.

tested clinically and they were classified according to the Classification of the American Heart Association.[2]

The three determinations, oxygen unsaturation, lactic acid content, and carbon dioxide combining power, were made in the same case after the same amount of work in the majority of cases. The oxygen unsaturation was determined at rest and approximately one minute after the exercise was completed, and the lactic acid and carbon dioxide combining power at rest and again four minutes after the exercise. The exercise consisted of climbing thirty feet in forty seconds. In a number of instances the determinations were made after double and triple the amount of work was performed.

The following changes were observed when the work consisted of climbing thirty feet in forty seconds: the 2A cases showed only slight changes, whereas the children classified as 2B showed, with few exceptions, a definite increase in the oxygen unsaturation and lactic acid, and a decrease in the carbon dioxide combining power. Several of the 2A and milder 2B cases were given double the amount of work. Of these children, the 2B cases manifested the greater changes. (Table I.) In five of the 2A cases it was necessary to triple the amount of work before definite changes were noted. Three cases

TABLE I.

Changes in O_2 Unsaturation, CO_2 Combining Power and Lactic Acid of Venous Blood After Exercise.

Cases	Class	No. of Determinations	Stairclimbing	Range of changes after exercise		
				O_2 Unsaturation Increase —Vol. %	CO_2 Combining Power Decrease Vol. %	Lactic Acid Increase mg. 100 cc.
5	2A	5	30 ft. in 40 sec.	0.17—0.64	0.08—1.15	1.8— 8.2
7	2B	15	30 ft. in 40 sec.	0.31—9.17	1.12—6.18	3.4—105.2
6	2A	7	60 ft. in 80 sec.		0.03—3.88	0.3— 7.0
4	2B	4	60 ft. in 80 sec.	3.61—6.83	4.41—6.8	11.1—122.8
5	2A	5	90 ft. in 120 sec.	0.89—4.93	3.44—5.12	9.4— 24.8

TABLE II.

Class	Stairclimbing	CO_2 Combining Power Increase—Vol. %	Lactic Acid Increase—mg. 100 cc.
2A	30 ft. in 40 sec.	1.45	7.6
2A	30 ft. in 40 sec.	2.70	11.6
2A	60 ft. in 80 sec.	1.59	0.3
2B	30 ft. in 40 sec.	3.80	—

[2] According to the Classification of the American Heart Association, Class 2 cases are patients with organic heart disease unable to carry on ordinary physical activity without discomfort; A, activity slightly limited; B, activity greatly limited.

classified as 2A, and one classified as 2B showed increases in the carbon dioxide combining power after exercise. (Table II.) We shall not attempt to explain these increases in the present report.

The results indicate that children whose tolerance for work is greatly diminished show greater changes in the oxygen unsaturation, lactic acid, and carbon dioxide combining power after performing measured amounts of work than children whose exercise tolerance is slightly diminished.

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Chemistry of the Lipoids of Tubercle Bacilli: XV. Water-soluble Sugars Obtained on Hydrolyzing Phosphatides from Human and Avian Tubercle Bacilli.*

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From the Department of Chemistry, Yale University, New Haven.

The phosphatide A-3 obtained from the human type of tubercle bacilli, strain H-37,[1] yielded on hydrolysis about 33% of water-soluble material, but when the preliminary analysis was published only 2 of the water-soluble constituents had been identified, *viz.*, glycerophosphoric acid and glucose. Mention was made in the former publication[1] that a slightly soluble phenylhydrazine derivative was obtained from the aqueous solution and this compound was regarded as a phenylhydrazine salt of a sugar acid. In addition, we also obtained a small amount of a colorless crystalline compound from the concentrated syrup. This unidentified substance was more specifically referred to in the paper dealing with the analysis of the phosphatide isolated from the avian tubercle bacilli.[2] We have recently examined more thoroughly the sugar fractions obtained from the phosphatide A-3 as well as those obtained from the phosphatide from the avian bacillus and we have been able to identify 2 other substances which are present in the hydrolysis mixture, *viz.*, mannose and inosite.

* The present report is a part of a cooperative investigation on tuberculosis, and it has been supported partly by funds provided by the Research Committee of the National Tuberculosis Association.

† Holder of a National Tuberculosis Association Fellowship at Yale University, 1929-30.

[1] Anderson, R. J., *J. Biol. Chem.*, 1927, lxxiv, 537.

[2] Anderson, R. J., and Roberts, E. G., *J. Biol. Chem.*, 1930, lxxxv, 519.

The separation of the water-soluble constituents was accomplished as follows: After hydrolysis with boiling dilute sulfuric acid the fatty acids were extracted with ether and the aqueous solution was freed of sulfuric acid quantitatively with barium hydroxide. After the solution had been filtered it was concentrated under reduced pressure, neutralized with barium hydroxide, and the barium phosphate and barium glycerophosphate were precipitated by adding alcohol and removed by filtration. A slight amount of barium in the filtrate was removed quantitatively with sulfuric acid and the solution was concentrated to a thin syrup. An excess of phenylhydrazine dissolved in a little alcohol was added when a crystalline derivative separated almost immediately. After the crystals had been filtered off the excess of phenylhydrazine was removed from the filtrate by treatment with benzaldehyde. The resulting precipitate was filtered off and the filtrate was extracted with ether. The solution was then concentrated to a syrup in a vacuum desiccator, when some colorless crystals separated. The crystals were freed from adhering syrup by washing with cold dilute alcohol and with 95% alcohol. The filtrate on concentration yielded a syrup which on treatment with phenylhydrazine hydrochloride and sodium acetate gave a good crop of glucosazone crystals.

The slightly soluble phenylhydrazine derivative, mentioned above, was recrystallized from hot 60% alcohol and it was obtained in the form of large dense colorless rhombic plates which were identical with crystals of mannose phenylhydrazone. The substance melted at 193-194° and there was no depression of the melting point when mixed with pure mannose phenylhydrazone. On analysis we found 10.45% of nitrogen, which is in close agreement with the calculated value, namely, 10.37%. The optical properties of the crystals have been compared with those of pure mannose phenylhydrazone by Dr. E. J. Roberts of this laboratory and the properties were found to be identical.

It appears to be well established, therefore, that one of the reducing sugars formed on hydrolyzing the phosphatides from the human and the avian tubercle bacilli is mannose.

The colorless crystalline compound which was isolated from the concentrated syrup has been found to be identical with the ordinary inactive inosite. The crude crystals, which contained a small amount of ash, were purified by several crystallizations from dilute acetic acid by adding alcohol and were obtained in the form of colorless prisms or needles characteristic of anhydrous inactive inosite. The substance gave the Scherer reaction and melted at

224-225° and there was no depression when mixed with some inactive inosite prepared from phytin. The values found on combustion agreed closely with the formula $C_6H_{12}O_6$. The optical properties of the crystals were examined by Dr. E. J. Roberts and were found to be identical with those of the ordinary inactive inosite. The identification of inosite may, therefore, be regarded as fully established.

Both inosite and mannose have been found widely distributed in plant and animal cells but this is the first time so far as we are aware that either substance has been observed as occurring in tubercle bacilli.

In addition to inosite and mannose the water-soluble syrup also contained a considerable amount of another reducing sugar which was believed at first to be ordinary glucose because it gave a good yield of typical glucosazone. The glucosazone, after it had been recrystallized from dilute alcohol, melted with decomposition at 205-206° and there was no depression of the melting point when the substance was mixed with pure glucosazone.

The syrup did not consist of pure glucose, however, because it was levorotatory and when heated with hydrochloric acid and resorcinol, a bright red coloration characteristic of ketoses was obtained. The rotation was determined on a solution containing 0.4603 gm. of the dried syrup in 25 cc. of water. In a 2 dm. tube the observed rotation was —0.38°; hence $[\alpha]^{22}_D = 10.3°$. It is probable, therefore that the residual syrup contained a mixture of glucose and fructose similar to invert sugar.

So far as we can judge by the results obtained, the 3 substances, mannose, inosite and invert sugar are present in the syrup from both phosphatide preparations in about equal amounts. We have no data to indicate in what manner these sugars are combined in the molecules of the phosphatides. It should be mentioned, however, that the phosphatides contain no free reducing sugar and it appears probable therefore that the simple carbohydrates which are obtained after complete hydrolyses are combined in the original molecules in the form of a polysaccharide which in turn must be combined either with fatty acids or with glycerophosphoric acid.

4778

Local Organ Hypersensitiveness: I. Experimental Production in the Rabbit Eye.

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(Introduced by W. W. Palmer.)

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Although there is a considerable and significant literature on the problems of general hypersensitiveness the known facts concerning local hypersensitiveness are few. The early work of Koch,[1] Calmette and Guerin,[2] and v. Pirquet[3] on the tuberculin reaction, the demonstration by Arthus[4] of skin necroses after repeated injection of foreign proteins, the work of Gay[5] on the typhoidin reaction and finally the work of Swift,[6] Dochez and Stevens,[7] and MacKenzie and Hanger,[8] and many others have helped our understanding of local changes in the reactivity of the skin. Furthermore, clinical observations have attested to the fact that apparently arrested areas of inflammation in various parts of the body have been relit by the introduction of the homologous noxious substance into distant sites. Experimental work along similar lines attempting to produce altered reactivity in other organs than the skin has not yielded uniformly positive results.

From a review of previous attempts to produce local hypersensitiveness it seemed that the critical requirement was the maintenance of a set of experimental conditions which would keep antigen in juxtaposition to body cells for a relatively long period of time. The structure of the anterior chamber of the eye presents a feature which probably allows this condition to be fulfilled. We had observed that heterologous erythrocytes when injected into the anterior chamber of the rabbit's eye would persist for several days. On this account the anterior chamber of the eye was the initial area chosen to test for the production of local hypersensitivity.

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[1] Koch, R., *D. M. W.*, 1891, xvii, 101 and 1189.

[2] Calmette, A., and Guerin, C., *Compt. rend. Soc. de Biol.*, 1908, lxiv, 722.

[3] v. Pirquet, C. E., *Ergb. d. inn. Med. u. Kinderheilk*, 1910, v.

[4] Arthus, M. M., *Compt. rend. Soc. de Biol.*, 1903, lv, 817.

[5] Gay, F. P., and Force, J. N., *Arch. Int. Med.*, 1914, xiii, 471.

[6] Swift, H. F., Derick, C. L., and Hitchcock, C. H., *J. Am. Med. Assn.*, 1928, xc, 906.

[7] Dochez, A. R., and Stevens, F. A., *J. Exp. Med.*, 1927, xlvi, 487.

[8] MacKenzie, G. M., and Hanger, F. M., Jr., *J. Immunol.*, 1927, xiii, 41.

A number of workers in ophthalmology have attempted to explain certain types of conjunctivitis and keratitis on the basis of "ocular anaphylaxis". Schieck,[9] Lemoine,[10] Tooker,[11] and Pasteur Vallery-Radot[12] among others have contributed clinical observations in this regard. Kodama[13] found that in sensitized guinea pigs the introduction of the shocking agent "into the orbit" produced stimulation of plain muscle, circulatory disturbances, and hypersecretion of eye glands. In attempting to explain the manifestations of sympathetic ophthalmia on the basis of "anaphylactic phenomena", Elschnig,[14] Kummel,[15] Wissman,[16] Fuchs and Meller,[17] von Szily,[18] and Woods,[19] have made certain contributions regarding the question of whether the injured uveal tract of one eye may act as a sensitizing and subsequent shocking antigen to the other eye. Wessely[20] found that the injection of horse serum into the cornea resulted in a local reaction which subsided in 48 hours. Two weeks later a spontaneous relighting of the same cornea appeared. If just prior to this response the cornea of the opposite eye was injected with horse serum, a violent reaction ensued in the injected eye. Stanculeanu and Nita[21] have demonstrated the Arthus phenomenon in the conjunctiva by the use of horse serum, and Kirchner[22] has sensitized the cornea of the rabbit to *S. scarlatinae* toxin, demonstrating sensitization by repeated injections into the cornea. Attempts to reactivate a locally sensitized eye by intravenous injection of the homologous antigen have been few and only successfully accomplished by Von Szily[18] and Riehm[23] in their studies on sympathetic ophthalmia. Schoenberg[24] injected human serum into the anterior chamber of 2 rabbits and tuberculin into the anterior chamber of 2 other rabbits.

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[9] Schieck, *Zeitschr. f. Augenheilk.*, 1914, xxxii, 95.

[10] Lemoine, *Arch. Ophth.*, 1929, i, 706.

[11] Tooker, C. W., *Arch. Ophth.*, 1929, ii, 540.

[12] Pasteur Vallery-Radot, *Presse Med.*, 1929, xxxvii, 529.

[13] Kodama, R., *J. Inf. Dis.*, 1921, xxviii, 48.

[14] Elschnig, A., *Arch. f. Ophth.*, 1911, lxxix, 428.

[15] Kummel, R., *Arch. f. Ophth.*, 1912, lxxxi, 486.

[16] Wissman, R., *Arch. f. Ophth.*, 1911, lxxx, 399.

[17] Fuchs and Meller, *J. Arch. f. Ophth.*, 1914, lxxxviii, 280.

[18] v. Szily, A., *Die Anaphylaxie in der Augenheilkunde*, Stuttgart, 1914. *Klinische Monatsb. f. Augenheilk.*, 1916, lvi, 79.

[19] Woods, A. C., *Arch. Ophth.*, 1917, xlvi, 8.

[20] Wessely, K., *M. M. W.*, 1911, lviii, 1712.

[21] Stanculeanu and Nita, *Compt. rend. Soc. de Biol.*, 1909, lxvi, 1112.

[22] Kirchner, O., *Z. f. Immunitatsfsch.*, 1928, lv, 157.

[23] Riehm, *D. M. W.*, 1929, lv, 907.

[24] Schoenberg, M. J., *N. Y. S. J. M.*, 1914, xiv, 493.

Intravenous injection of the homologous anigen 2 weeks later produced no significant eye reaction. Brown and Dummer[25] injected increasing doses of hemolytic streptococcus vaccine into the conjunctiva of 2 rabbits for 4 days and then injected a suspension of the living organisms intravenously on the following day. No eye reactions were noted.

A preliminary experiment to produce local hypersensitivity in the eye was carried out as follows: Two-tenths of a cubic centimeter of the anterior chamber fluid from the right eye of 5 rabbits was removed under cocaine anesthesia and replaced by 3 separate substances. Each of 2 animals received 0.15 cc. of a 40% saline suspension of guinea pig red blood cells, 2 received a similar quantity of a 40% saline solution of fresh egg white and the remaining animal 0.15 cc. of physiological saline solution. There was a very slight injection of the conjunctival vessels for 24 hours in the saline treated animal. In the other 4 animals the anterior chamber appeared cloudy and definite injection of the iris and conjunctiva occurred, which cleared up completely in from 3 to 6 days. The eyes of all animals remained normal in appearance until the thirteenth day after injection, when a test for the local sensitivity of the eye was attempted. Rabbit 1, sensitized with guinea pig red blood cells, was injected intravenously with 1.0 cc. of 30% saline solution of fresh egg white, while Rabbit 2, similarly sensitized, was injected intravenously with 1.0 cc. of a 40% suspension of guinea pig red blood cells. In a similar manner, of the 2 animals prepared by an

TABLE I.
Reactivation of Locally Sensitized Eyes.

Rabbit No.	Right eye sensitized with	13 days later injected intravenously with	Maximum reaction in eye 5 hours later
1	Guinea pig red blood cells	Egg albumen	None
2	Guinea pig red blood cells	Guinea pig red blood cells	Hyperaemia of conjunctiva and iris. Chemosis, lacrimation
3	Egg albumen	Guinea pig red blood cells	None
4	Egg albumen	Egg albumen	Hyperaemia of conjunctiva and iris. Chemosis, lacrimation
5	Physiological saline	Guinea pig red blood cells	None
		24 hours later Egg albumen	None

[25] Brown, A. L., and Dummer, C., *Arch. Ophth.*, 1929, ii, 573.

injection of egg white into their anterior chambers, one was given egg white and the other guinea pig red blood cells intravenously. The saline control animal was injected with 1.0 cc. of guinea pig red blood cells and 24 hours later with 1.0 cc. of egg white. The animals injected with their homologous antigens reacted positively while the remaining animals failed to show any change in the eye.

The positive reaction in the eye was characterized by a moderate to deep injection of the conjunctival vessels, a similar though slight hyperaemia of the iris, slight chemosis and moderate lacrimation. This reaction tended to reach a maximum in about 5 hours and faded in the course of about 24 hours. The results of this experiment, shown in Table I, signify that specific local hypersensitiveness can be produced in the rabbit eye. Subsequent intravenous injections of the homologous antigens resulted in a much decreased local reaction. This we interpreted as a desensitization phenomenon. This desensitization could be completely accomplished by repeated injection of the usual doses of the homologous antigen.

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Local Organ Hypersensitiveness: II. Repeated Response in the Rabbit Eye.

DAVID SEEGAL AND BEATRICE CARRIER SEEGAL.
(Introduced by W. W. Palmer.)

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The demonstration of the availability of the rabbit's eye for local sensitization by a single antigen[1] suggested the possibility of the production of repeated eye response under appropriate experimental conditions. In order to avoid the phenomenon of desensitization and to work with an eye which might be kept more or less constantly in a condition of sterile inflammation, a "multiple" antigen was used to sensitize the eyes of a new series of rabbits. This was prepared by mixing together the citrated or defibrinated blood of a number of animals along with some other foreign proteins. The first multiple antigen consisted of citrated guinea pig, sheep and pigeon blood, horse serum, 5% casein, 5% egg white, and an anhemolytic streptococcus vaccine. Considering each blood as com-

[1] Seegal, D., and Seegal, B. C., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii,

posed of 2 antigens, serum and red cells, this constituted a total of 10 different antigens. One anterior chamber, or in some cases both anterior chambers, were injected with 0.2 cc. of this mixture after the removal of 0.2 cc. of anterior chamber fluid, as previously described.[1] The local sensitiveness of the eyes was then tested by the separate intravenous injection of each ingredient of the multiple antigen, after the initial reaction in the eye, due to the presence of the foreign proteins, had subsided. The sensitized eye responded to each succeeding injection by a sterile inflammatory reaction already described. The time allowed to elapse between the intravenous injections of each of the separate antigens varied from one to several days. The experimental results obtained in 30 rabbits over a period of 11 months may be summarized as follows:

1. Two tenths of 1.0 cc. of a multiple antigen containing 10 separate ingredients, or in other words, 0.02 cc. of a single foreign protein, when introduced into the rabbit's anterior chamber, is sufficient to produce an altered reactivity of that eye such that when 1 cc. of one of the 10 antigens is introduced intravenously the eye shows hyperaemia of the iris and conjunctiva with more or less oedema and lacrimation during the next 24 hours.

2. Eyes sensitized with the multiple antigen will show an inflammatory reaction for as long as 8 months, at least, following the intravenous injection of fractions of the total antigen.

3. Repeated daily intravenous injections of a single antigen usually produce no reaction after the first few days. Injection of different antigens intravenously on succeeding days produces a continued sterile inflammatory process in the sensitized eye. After the total number of single antigens has been injected, repetition of these injections now fails to produce a similar response. Instead, the eye reaction is at a much lower level and the inflammatory response is manifested only to a few of the antigens injected intravenously.

4. Permanent desensitization of the eye has not occurred in animals which have been followed for at least 8 months. Animals may develop maximal eye responses following intravenous injections of the same antigen if sufficient time has elapsed between injections. Nevertheless, the eye reactions which can be elicited 6 or 7 months after sensitization are less intense than the initial responses.

5. The ability of the eye to light up following the intravenous injection of the homologous antigen is not due to an initial tissue injury as is proven by the fact that the reaction is specific and anterior chambers injured with typhoid vaccine will not respond sub-

sequently when the various proteins used for sensitization of the other eyes are injected intravenously.

6. It has been impossible to demonstrate sensitivity in the eye by the intravenous shocking route until at least the fifth day following introduction of the antigen into the anterior chamber.

7. Rabbits vary considerably in the intensity of reaction which can be elicited from them, but none was found which failed completely to give any reaction.

8. An experiment may be reported in which an eye sensitized to a multiple antigen containing cat red blood cells became inflamed 5 hours after the introduction of 35 cc. of a 50% suspension of partially hemolyzed cat red blood cells by stomach tube into the gastro intestinal tract.

9. Experiments in progress show that toxic antigens and bacterial bodies may be used to elicit the type of reaction obtained with the multiple antigen described above.

4780

Observations on the Blood Vessels of the Vascular Membrane of Chicken Embryos.

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It was the object of these experiments to attempt to discover first, whether there was a difference in innervation between the blood vessels of the vascular membrane of chicken embryos and innervated blood vessels; and second, what changes occurred in the blood vessels of the embryos' vascular membrane during the course of its development. The experiments were carried out in a constant temperature room with eggs at all ages but especially at the 3rd and 4th days of incubation. The eggs were opened at the end of the air-chamber. In connection with innervation both physiological and anatomical investigations were made. In the physiological experiments stimuli of mechanical, electrical and chemical varieties were applied. In the manner of Ricker it was found possible to grade the strength of the stimuli into weak, medium and strong, and doses were found of each variety of stimulus so that comparable effects in the vessels could be observed: constriction or dilatation, but more often dilatation with the weak stimuli, constriction with

medium stimuli, and dilatation with the cessation of flow, with strong stimuli. It was characteristic of all these effects, except perhaps those with strong stimuli, that *only* the vessel or vessels in the immediate neighborhood of the stimulus was affected. The stimulus did not spread to surrounding areas. Adrenalin behaved differently from the case of innervated vessels, for with small doses (0.1 ccm. of a 1% solution) no effect on the vessels was observed. With doses 4 times as great, one-half to 4 or 5 minutes were required to bring about contraction. In such cases a like amount of salt solution had the same effect.

In the anatomical experiments sections were made of embryos 3, 4 and 7 days old, and these were stained both by Bielschowsky's method and by the method of methylene blue reduced by rongalit. In each case tissues in which it was known that nerves were present were prepared in the same solutions. Nerves were found always in the controls and never in the vascular membrane. It appears, therefore, that here is genuinely a tissue free from nerves. In another place there will be described the physiological consequences of this fact.

In the experiments dealing with change in these vessels in the course of development, it was found that where the same stimulus was applied the reaction was the same provided the size of the vessel stimulated was the same. It made no difference what was the age of the embryo. A larger vessel we found was less irritable than a small one and capillaries were most irritable of all. There are naturally larger vessels in larger embryos, but as has been said, the larger the vessel the less the irritability. In none of the vessels of the vascular membrane were elastic fibers found nor was there found at any period of development evidence of degeneration. It appears, therefore, that these vessels at the end of the period of incubation are discarded without having passed through a process of growth and of evolution.

4781

Reduction Intensity of Sterile Bouillon: Dye Reduction Controlled by Electrode Measurements.*

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The reduction potential which may be attained under anaerobic conditions in sterile bouillon has been reported[1] as reaching an apparent equilibrium at E_H—0.085 to 0.095 volt at pH 7.5.

Dubos[2] has found, however, that the reduction intensity of sterile broth is sufficient to bring about the reduction of dyes more positive in Eo value than indigo disulfonate, and at least the partial reduction of this dye. This corresponds to a potential considerably more negative than those reported for electrode measurement. Simultaneous measurement of dye reduction and electrode potential is necessary to explain the discrepancy and to throw light on the possible catalytic effect of the dyes. This is the subject of the present paper.

The procedure for measurement of the electrode potentials has been described elsewhere.[1] In the present work a quadrant electrometer was used instead of a galvanometer in order to minimize polarization of the electrodes. Sterility of the bouillon was maintained throughout. The bouillon represented various lots of the infusion medium used in this laboratory for growth of the streptococcus. It was adjusted to pH 7.6, buffered by phosphate in M/10 concentration and autoclaved immediately before use in each experiment. Determination of the final pH was made with the hydrogen electrode at the close of the experiment, in some cases.

The time-potential course of the bouillon without addition of dye was followed first for a period of one or more weeks. The potential was regarded as having attained its equilibrium value when no further negative drift was observed over a period of at least 48 hours. At this point a sufficient amount of the indicator dye in deaerated solution was added through an anaerobic burette to give a distinct color to the bouillon, and the subsequent course of the electrode potentials was followed until again a value was reached which remained constant for 48 hours or longer. Methylene blue, indigo tetra-, di-, and mono-sulphonate, obtained from the La Motte

* This work was aided by a grant from The Chemical Foundation, Inc., N. Y. City.

[1] Coulter, C. B., *J. Exp. Med.*, 1929, xlix, 711.

[2] Dubos, R., *J. Exp. Med.*, 1929, xlix, 507.

Chemical Co., were the dyes employed; their concentration in bouillon was between 0.0001 and 0.0002 M.

The apparent equilibrium potentials of the bouillon in this series, before the addition of the indicator, have fallen between E_H —0.110 and —0.0135 volt within the pH range of 7.2 to 7.45. After the addition of the dye, the final electrode potentials have attained E_H —0.135 to —0.150 volt, so that there is evident a distinctly more negative potential attained in the presence of the dye and apparently catalyzed by it. The oxidation reduction system of the bouillon itself, however, determines the behavior of the electrodes since the dyes of increasingly negative Eo values do not occasion a correspondingly greater change in the potential of the bouillon.

All the dyes were completely decolorized except mono-sulphonate and with this dye only a faint greenish tint was observed. The percentage reduction of each of the 4 dyes, calculated for the pH and redox potential found in the bouillon in the corresponding experiment is as follows:

M. B.	100%	reduction
Tetra-sulf.	98	"
Di-sulf.	34	"
	47	"
Mono-sulf.	8	"
	14	"

The slow precipitation of the mono-sulphonate may explain the very faint color present under conditions which should yield only 8 or 14% reduction. The case of the disulphonate is of more importance since the pioneer observations of Smith[3] and those of Dubos record reduction of this dye by sterile bouillon. In our experiments the decolorization of the dye has been complete within 48 hours while the observed potentials even at the most negative value attained correspond to only partial reduction. Even under the most rigid protection from oxygen this dye appears to combine with a constituent of the bouillon: the blue color was not restored at the conclusion of the experiments by aeration or the addition of ferricyanide. Peculiarities noted by Clark in his study of the disulphonate led this author to suspect the formation of a compound between the reduced form of the dye and other substances. The conditions in our experiments were such as to favor the formation of such a compound.

[3] Smith, T., *Cent. f. Bakt.*, Orig., 1896, xix, 181.

4782

Some Effects of Denervation on Muscular Contraction.

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The effect of denervation on contraction of the gastrocnemius of the frog was observed by recording, simultaneously, the myograms of the intact and denervated muscle. Denervation was accomplished by excising 5 to 8 mm. of sciatic nerve from one leg (7 to 21 days prior to the experiment). Thirty experiments were made on winter frogs *(Rana pipiens)*. A gastrocnemius-sciatic preparation was made from the left (operated) and right (intact) legs. Both preparations were then mounted in such a way that the intact nerve-muscle and its writing lever were directly below the denervated muscle and in alignment with its writing lever. The muscles were stimulated by a maximal tetanus, simultaneously, through their nerves, by means of a Harvard inductorium. Stimulation was either by a switch, operated by hand or, when recurrent automatic stimulation was desired, by means of a switch placed on the kymograph and operated by its rotation. An electromagnetic signal placed in the primary circuit and arranged to write upon the drum in alignment with the two muscle levers, recorded the number and duration of the stimulations.

The myograms of denervated muscle present certain peculiar differences from those of normal muscle. The initial speed of contraction as indicated by the steepness of ascent of the lever, is greater in denervated than in intact muscle. All of the records agree in this particular. This speed factor should result in a diminished latent period for the denervated muscle. In these experiments, however, the technique was not sufficiently refined to demonstrate this difference. The relaxation phase also takes place more quickly than in the intact muscle. The difference is especially marked at the end of the relaxation phase. There appears also to be a shortening of the intermediate phase, or crest of the myogram, although this fact is less easy to demonstrate than the other two. The total duration of the myogram is, therefore, considerably less in

* The experiments on frogs were performed in the Zoological laboratory of Oberlin College. Grateful acknowledgment is made to Profs. Buddington and Rogers, who kindly placed materials and laboratory facilities at my disposal. The experiments on cats and further experiments on frogs are being carried on in the H. K. Cushing Laboratory of Experimental Medicine.

the denervated than in the normal muscle. Staircase phenomenon is marked in the intact muscle and is usually absent or very slight in the denervated muscle.

The experiments permit the suggestion that these changes may be due to altered viscosity of the denervated muscle. J. F. Fulton[1] has proposed a similar explanation on the ground of the diminished tension and the increased area of the myograms showing the staircase effect.

Further investigation of these changes in muscle, on frogs and cats, is in progress. Preliminary observations on cats, in which the left sciatic was sectioned, indicate that the elasticity of the denervated muscle, as measured by the stretch produced by equal increments of load, is less than that of intact muscle subjected to the same experimental conditions.

4783

Production of a Premenstrual Endometrium in Castrated Monkeys by Ovarian Hormones.*

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From the Department of Zoology of the University of Wisconsin.

The several theories explaining menstrual phenomena in primates for the most part agree that it depends upon hormonal function of the ovaries. Some authors emphasize follicular hormone, some corpus luteum, while others believe that both are concerned. Van Herwerden[1] found that in *Cercocebus cynomolgus* menstruation may occur without ovulation and Corner[2] and Allen[3] have established the same fact for *Macacus rhesus*. Allen also discovered that rather scanty menstruation in castrate and sexually immature monkeys usually followed after a certain degree of uterine growth had been induced by the injection of follicular hormone. These authors agree, however, that the uterine endometrium under these conditions is not typical of the normal premenstrual endometrium found only

[1] Fulton, J. F., "Muscular Contraction and the Reflex Control of Movement," 1926, 252.

* Assisted in part by a grant from the National Research Council, Committee on Problems of Sex.

[1] Van Herwerden, M., *Monatschr. f. Geburts u. Gynaek.*, 1906, xxiv, 730.

[2] Corner, G. W., *Contributions to Embryology*, 1923, No. 332, 75.

[3] Allen, E., *Contributions to Embryology*, 1927, xix, No. 380, 1.

when a corpus luteum is present. Novak[4] suggested that the physiology of menstruation could perhaps be solved if follicular and corpus luteum extracts of known potency were administered to experimental animals in the same sequence that they normally occur in the menstrual cycle.

Allen[3] has shown that physiologically active preparations of the follicular hormone do not promote typical premenstrual development of the uterine endometrium. We have made corpus luteum extracts which, in proper combination with follicular hormone, produce many physiological reactions ascribed to the normal corpus luteum (Hisaw,[5] Weichert,[6] Hisaw *et al*[7]). This paper reports the experimental use of these corpus luteum preparations on the production of premenstrual development of the uterine endometrium of castrate *Macacus rhesus* monkeys.

Five sexually mature female monkeys were castrated. They were first brought into full oestrum by the injection of follicular hormone (kindly furnished by E. R. Squibb & Sons) and then given a series

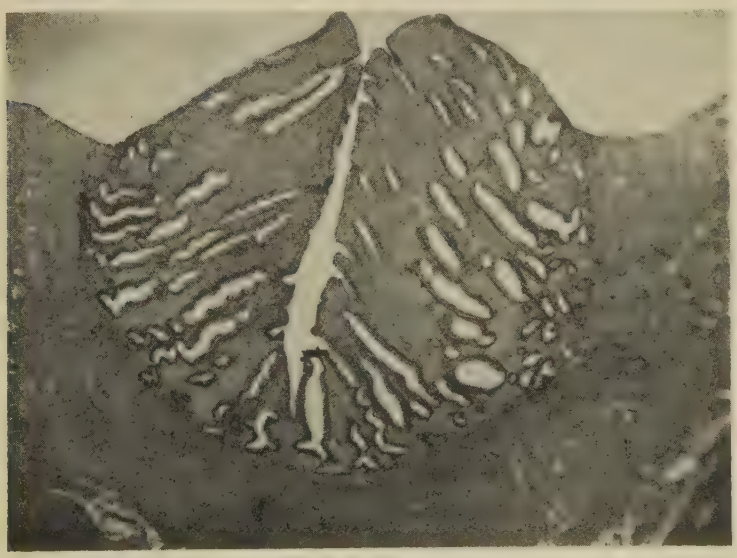

FIG. 1.

Monkey 4. Section through the lateral wall of the uterus of a castrate monkey which had received 340 units of follicular hormone followed by corpus luteum extract equivalent to 1040 gm. of fresh tissue.

[4] Novak, E., *J. Am. Med. Assn.*, 1928, xc, 339.

[5] Hisaw, F. L., *Physiol. Zool.*, 1929, ii, 59.

[6] Weichert, C. K., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 490.

[7] Hisaw, F. L., Fevold, H. L., and Meyer, R. K., *Physiol. Zool.*, 1930, iii, 135.

of injections of corpus luteum extracts. Monkeys 4 and 5 received the largest dosage of corpus luteum extract and showed the best development of the uterine endometrium. These 2 animals received the following treatment:

Monkey 4 (Fig. 1). Experiment began 14 days after castration, received 340 rat units of follicular hormone during the next 10 succeeding days. This was followed immediately by the injection of corpus luteum extract for 7 days which totalled the equivalent of 1040 gm. of fresh corpus luteum tissue. The animal was sacrificed on the seventeenth day.

Monkey 5 (Fig. 2). Castrated 13 days after last menstruation. Right ovary contained a very large follicle while the left ovary had only small follicles and there were no corpora lutea in either ovary. This animal was used in the experiment immediately after castration and during the next 11 days received 305 rat units of follicular hormone. On the ninth, tenth, and eleventh days she was given 10, 10, and 5 rat units of follicular hormone in combination with a daily dose of corpus luteum extract equivalent to 260 gm. of fresh tissue. The corpus luteum treatment was continued for an additional 4 days, at the end of which time a total equivalent to 1690 gm. of fresh corpus luteum tissue had been used. The animal was sacrificed at the end of the sixteenth day.

FIG. 2.

Monkey 5. Same as Fig. 1, except 305 rat units of follicular hormone and corpus luteum extract equivalent to 1690 gm. of fresh tissue were given.

We do not know whether or not the dosages used were excessive, but it should be mentioned that monkeys 1, 2 and 3 received corpus luteum extract equivalent to 360, 400 and 450 gm. respectively and while showing some modification of the endometrium were not nearly so favorable as animals 4 and 5, whose protocols are given. The accompanying figures do show, however, that it is possible to produce premenstrual changes in the uterus of castrate monkeys through the combined use of follicular and corpus luteum hormones.

4784

Physiology of Corpus Luteum VII. Maintenance of Pregnancy in Rabbit After Very Early Castration, by Corpus Luteum Extracts.

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From the Department of Anatomy, University of Rochester, School of Medicine and Dentistry.

In a recent series of papers[1] we have described the preparation and effects of an extract made from the *corpora lutea* of swine. When administered to recently spayed adult rabbits, or to immature rabbits whose uteri have been brought to the mature resting state by injections of oestrin, the *corpus luteum* hormone (progestin) induces alterations of the endometrium characteristic of pregnancy. If female rabbits are mated and castrated 18 hours later, while the fertilized ova are still in the Fallopian tube, the extracts substitute for the removed ovaries so completely that the embryos are nourished, become implanted, and develop in the uterus exactly as in normal pregnancy under the influence of the mother's own *corpora lutea*. In the third of our previous reports we have described 2 animals which were carried to the 13th and 19th days of pregnancy respectively. The demonstration of normal implantation with normal foetuses in these animals at autopsy led us to attempt to carry other animals through the full term, with the results now to be reported.

The procedure in general was as follows: A doe was mated, usually to 2 bucks, and was subjected to bilateral double oöphorectomy 18 hours after mating. The number of ruptured follicles in each ovary was noted. Administration of the extract by subcu-

[1] Corner, G. W., *Am. J. Physiol.*, 1928, lxxxvi, 74; Corner, G. W., and Allen, W. M., *Ibid.*, 1929, lxxxviii, 326; Allen, W. M., and Corner, G. W., *Ibid.*, 1929, lxxxviii, 340.

taneous injection was begun on the day of operation and was continued daily thereafter. The preparation used was the crude oily extract described in our second paper. About the 8th to the 12th day of the experiment the animal was again explored under ether anesthesia, for the purpose of determining whether or not implantation had taken place. This procedure enabled us to conserve the costly extract in case the pregnancy had not continued. If the animal was found to be pregnant the experiment was continued, until terminated by spontaneous delivery or by a third laparotomy, as indicated below. In those experiments in which pregnancy did not continue to full term the extract was again tested on another rabbit by the test given in our previous paper (No. 2 of the series).

Four animals which received from 0.2 cc. to 0.5 cc. daily of an extract known to be fully potent, went to spontaneous delivery on the 29th, 32nd, 28th and 32nd day respectively (usual duration of normal pregnancy in our colony 33 days). Two of these animals received the extract daily throughout the whole experiment, and 2 received it for 21 days only. This should not, however, be taken to demonstrate that the hormone is unnecessary during the last week of pregnancy in the rabbit; the extract used in these experiments is a heavy oil which is absorbed very slowly, and therefore the 2 animals which received only 21 injections may have possessed subcutaneous stores sufficient to carry them during the last week.

Two animals which received respectively 1 cc. and 0.5 cc. of a potent extract daily throughout the whole term failed to give birth to their young on the 33rd day. By means of Caesarian section on the 34th and 36th days respectively, fully developed dead foetuses were found *in utero*, together with others already undergoing resorption. From the size of the better-preserved foetuses and from other signs it appeared that in one case the young had grown until the 26th day, in the other until the 32nd or 33rd day.

In 8 animals the embryos did not survive. In 5 of these cases no implantation occurred, and in 2 others the embryos became implanted but were resorbed soon after. In all of these cases the extract when subsequently tested was discovered to have lost potency.

Another animal which received a potent extract in adequate dosage, did not implant her embryos at all, as demonstrated by exploration on the 9th day; this was the only unexplained failure in the series.

These experiments demonstrate that our crude extract of pigs' *corpora lutea* is able, when administered in a fully potent condition

and in adequate dose, to maintain pregnancy in rabbits, spayed at the 18th hour after mating, until full term.

Experience obtained during the course of this work enables us to state certain precautions which must be used in similar experiments. We find that the crude extract does not always maintain its potency longer than one week when kept in the oily state, and therefore we now keep our stock of extract dissolved in 95% ethyl alcohol, distilling off weekly a supply for immediate use. The dosage necessary to insure implantation and maintenance of pregnancy is larger than that required to produce progestational proliferation according to our standard test of potency. Usually 0.1 cc. of the crude extract daily for 5 days, or a total of 0.5 cc., contains one rabbit unit; but to insure maintenance of pregnancy 0.5 cc. should be given daily. In all cases in which pregnancy terminates prematurely, the extract used should be tested for potency on another rabbit.

4785

Influence of Tyramine on the Number of Red Corpuscles in the Circulating Blood.

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Rabbits were given tyramine subcutaneously in single doses of various sizes, in hourly doses and in daily doses. With but few exceptions the effect was to increase the number of red corpuscles per cubic millimeter in the circulating blood. The hydrochloride was used. This was dissolved in 0.9% sodium chloride solution in such proportion that 1 cc. contained the dose per kilo of body weight, and the solution warmed to body temperature before injection.

Single doses of from 0.1 mgm. to 20.0 mgm. per kilo of body weight were used. The average results of these are given in Table 1. A dose of 0.5 mgm. per kilo caused an increase in the red blood corpuscle count of 18.46% and was selected as an average effective dose.

Each of 4 rabbits was given 0.5 mgm. per kilo of body weight and the dose repeated at intervals of one hour until 4 doses had been given. The results are summarized in Table II.

TABLE I.

Dose in mgm. per kilo.	Number of observations	Initial count	Maximal count	Maximum in	Percentage increase	Exceptions
0.1	4	6,033,000	6,253,000	30 min.	3.64	
0.2	5	5,247,000	5,493,000	30 "	4.68	Decrease in one
0.3	5	6,064,000	6,204,000	48 "	2.30	Decrease in two
0.4	5	5,778,000	6,501,000	30 "	12.51	Decrease in one
0.5	5	5,568,000	6,596,000	48 "	18.46	
0.6	5	6,475,000	7,377,000	42 "	13.93	
0.8	5	6,232,000	7,510,000	42 "	20.50	
1.0	7	6,085,000	6,670,000	47 "	9.61	Decrease in one No change in one
2.0	4	5,626,000	6,682,000	30 "	18.77	
5.0	4	5,870,000	6,793,000	30 "	15.72	
10.0	3	5,262,000	7,078,000	30 "	34.51	
20.0	2	6,500,000	8,920,000	60 "	37.23	

TABLE II.

Dose: 0.5 mgm. per kilo of body weight.

Rabbit number	Initial count	One hour after one dose	One hour after 2 doses	One hour after 3 doses	One hour after 4 doses	2 hours after 4 doses	3 hours after 4 doses
51	6,026,000	7,184,000	7,968,000	8,300,000	6,760,000	8,026,000	6,864,000
52	5,540,000	6,340,000	6,020,000	6,240,000	6,160,000	7,448,000	5,820,000
54	5,220,000	5,840,000	5,920,000	6,320,000	6,120,000	5,900,000	5,460,000
59	5,520,000	6,064,000	6,208,000	6,720,000	6,140,000	7,600,000	7,410,000
Average	5,576,000	6,357,000	6,529,000	6,895,000	6,295,000	7,243,000	6,388,500
Percentage increase		14.00	17.09	23.65	12.89	29.89	14.56

Twelve rabbits, 15 weeks old, were divided into 3 groups of 4 each. Two groups received tyramine and the third served as a control. The tyramine was given daily for 2 weeks, then 3 times a week for 2 weeks, then daily for 2 weeks, and finally 3 times a week for 2 weeks. The dose for one group was 0.5 mgm. of the hydrochloride per kilo of body weight and for the other 5.0 mgm. per kilo. At the same time the control rabbits were given a subcutaneous injection of an equal quantity of 0.9% sodium chloride solution. Living conditions were the same for all. The red corpuscles were counted twice each week and the body weight was noted once a week. The accompanying figure shows the results obtained.

At the end of 8 weeks 2 rabbits from each group were killed by chloroform and sections of the bone marrow made. These were prepared and studied by the departments of pathology and histology. They reported that the 2 rabbits from the 5.0 mgm. group showed very active proliferation of red corpuscles, the 2 from the control group and one from the 0.5 mgm. group showed moderate activity, and the remaining one from the 0.5 mgm. group showed the least.

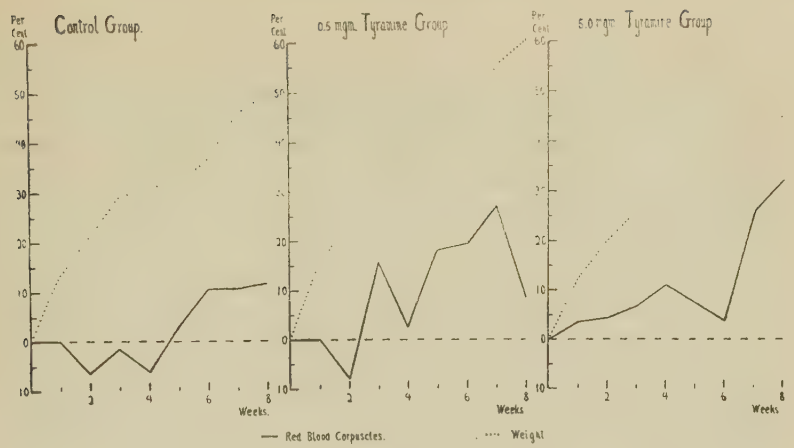

FIG. 1.

Conclusions: 1. The subcutaneous administration of tyramine hydrochloride to rabbits in amounts from 0.1 mgm. to 20.0 mgm. per kilo of body weight was followed by an increase in the number of red corpuscles per cubic millimeter in the circulating blood. 2. The repeated subcutaneous administration of a fixed amount of tyramine hydrochloride to rabbits was accompanied by a progressive increase in the number of red corpuscles per cubic millimeter in the circulating blood. The results in these experiments resemble those obtained when a secretin preparation was administered to rabbits (Downs and Eddy[1, 2]).

4786

Amylase Studies in Dogs Sera Following Ligation of the Pancreatic Ducts.

CARL E. JOHNSON AND CARL H. WIES. (Introduced by Raymond Hussey.)

From the Departments of Pathology and Surgery, Yale School of Medicine, New Haven, Connecticut.

Numerous investigators have studied the amylase concentration in normal dog's blood stream using various methods. The methods most commonly used are: the starch iodide of Wohlgemuth; the change from starch to sugar as indicated by copper reduction; the

[1] Downs, A. W., and Eddy, N. B., *Am. J. Physiol.*, xliii, 415.
[2] Downs, A. W., and Eddy, N. B., *Am. J. Physiol.*, xlvi, 209.

viscosimetric method of enzyme activity of Northrop and Hussey. Workers with these methods have not established one definite level in absolute units for the amylase concentration in the sera of healthy animals, but each has found that a normal range can be established with his own particular method.

Elman and McCaughan, using the viscosimetric method found that the normal concentration in dogs fell between 1.5 and 2.5 arbitrary units. Fasting or diet did not alter this range. The writers, using the same method with a more precise technique, found a normal range slightly higher, *i. e.*, 6 to 13 units. A unit being the amount of enzyme which would produce a 20% change in outflow time of a 7% starch solution in a modified Ostwald viscosimeter in one hour, at a pH of 7.6 and a temperature of 37.50° C. ± 0.02°.

In a series of experiments using 4 dogs with suitable controls, the amylase concentration was studied, after ligation of all the pancreatic ducts. In these experiments 2 dogs were used. Each was kept under the same conditions with respect to feeding and general care and given the same operative procedure except that all the pancreatic ducts in one were ligated. The dog to be used as the control animal was chosen by lot, the remaining dog being the one for the ligation. It was found that amylase concentration of the serum increased from 9 to 20 times normal following the operation in the dogs whose pancreatic ducts had been tied, to reach a maximum on the third day postoperative. Following this there was a gradual decline to approximately normal on the twelfth day after operation. The experiments covered periods varying from 14 to 90 days. Histological studies of all these animals have been made and will be reported subsequently.

4787

Composition of the Cell Sap of Halicystis Ovalis (Lyng.) Areschoug.*

S. C. BROOKS.

From the Department of Zoology, University of California, Berkeley, California.

The cell sap of a species of *Halicystis* which "is cast up on the beaches as clear green, balloon shaped cells" at Bermuda was analyzed by Osterhout and Dorcas[1] and reported to differ strikingly from that of *Valonia macrophysa* Kütz, collected at Bermuda, as determined by Wodehouse[2] and by Osterhout.[3] The contrast seemed all the more striking since the species was at that time erroneously identified as *Valonia ventricosa* J. G. Ag. This identification was subsequently revised by Blinks,[4] who considers the species analyzed by Osterhout and Dorcas to be *Halicystis ovalis* (Lyng.) Areschoug.

Osterhout and Dorcas[1] reported that while cells of *Valonia macrophysa* contain a sap which is very rich in potassium and poor in sodium, precisely the opposite condition obtains in *Halicystis* (which they call *Valonia ventricosa*). The ratios of potassium to sodium in the two forms were found to be 5.72 and 0.0278 respectively. Since the cells which they analysed floated in sea water while normal *Valonia* cells of either species sink,[5] it appeared questionable whether these cells were entirely normal. Osterhout and Dorcas[1] say in this connection: "The writers made every effort to obtain young growing cells. . . . . During the past 2 years a few cells apparently of the same species have been found attached. . . . . Unfortunately there seems to be no prospect of obtaining such cells in sufficient quantity for analysis." The analysis reported by them refers to cells torn loose from their habitat and drifted ashore.

Blinks[6] has recently reported that the electromotive forces developed by cells of *Halicystis* differ strikingly from those previously

* The expense of this research was met in part by a grant from the Board of Research of the University of California, for which grateful acknowledgement is made. I am also greatly indebted for the facilities made available by the Hopkins Marine Station, Pacific Grove, California, and the Division of Plant Nutrition of this University.

1 Osterhout, W. J. V., and Dorcas, M. J., *J. Gen. Physiol.*, 1925, vii, 633.

2 Wodehouse, R. P., *J. Biol. Chem.*, 1917, xxix, 453.

3 Osterhout, W. J. V., *J. Gen. Physiol.*, 1922, v, 225.

4 Blinks, L. R., *Science*, 1927, lxv, 429.

5 This has repeatedly been observed by M. M. Brooks and by the writer.

6 Blinks, L. R., *J. Gen. Physiol.*, 1929, xiii, 223.

observed with *Valonia*. He states: "The sap of the small cells used in these experiments was essentially the same in composition as that of the large floating cells" as previously reported by Osterhout and Dorcas.

It is not made clear whether this statement is based on more than qualitative tests, and it seems probable, in the absence of any definite statement, that the cells used were those cast up on the beaches.

It therefore seems worth while to publish exact determinations of the potassium and sodium content of the sap of undoubtedly normal cells of *Halicystis* collected from their actual positions of growth on *Lithothamnion* incrustations below mean low tide level on the Pacific Coast. These are given below in Table I, together with similar data for *Valonia macrophysa* and *Valonia ventricosa* collected at Tortugas, and those for *Halicystis* as given by Osterhout and Dorcas.[1] The methods used in the new analyses reported here were like those described by Brooks,[7] except that potassium was determined gravimetrically as the chloroplatinate. The analyses were made by Mr. Roy Overstreet.

TABLE 1.

The potassium and sodium content of sea water and sap from various sources expressed as per cent of the total halide content in each case. The sap analyses made by ourselves are those of composite samples obtained by extracting sap from a large representative group of cells.

Solution	K	Na	K:Na	Author
Sea water. (Bermuda)	2.15	85.87	0.0251	Osterhout and Dorcas[1]
Halicystis, Sap, (Bermuda)	2.58	92.80	0.0278	" " "
Sea water, (Pacific Coast)	2.17	30.1	0.0271	Brooks, original
Halicystis, sap, (Pacific Coast)	58.9	39.2	1.50	" "
Valonia macrophysa sap (Tortugas)	79.2	14.3	5.53	Brooks[7]
Valonia ventricosa sap (Tortugas)	92.1	8.7	10.58	Brooks, original

It will be seen that normal *Halicystis* cells of the Pacific coast form, which appear to be morphologically identical with *H. ovalis*,[8] contain a sap much more like that of the 2 species of *Valonia* than like that of *Halicystis ovalis* as reported by Osterhout and Dorcas. It is true that these *Halicystis* plants do not accumulate potassium to quite so high a concentration as do the Valonias, nor contain so little sodium, but they do contain more potassium than sodium (ratio 1.50:1), and accumulate potassium, while in effect excluding sodium. Thus potassium is 28.12 times as concentrated in the sap as

[7] Brooks, S. C., *Protoplasma*, 1929, viii, 389.

[8] See, for example, Collins, F. S., "The Green Algae of North America." Tufts College Studies (Scientific series), 1909, II, No. 3, p. 372.

in the surrounding sea water, while sodium is only 0.517 times as concentrated. The corresponding accumulation ratios (concentration in sap : concentration in sea water) reported by Osterhout and Dorcas for drifted specimens of what is supposedly the same species are 1.2 for potassium, and 1.08 for sodium.

This difference in composition of the sap of plants collected in the places and manners described corresponds with the fact that Osterhout and Dorcas found their *Halicystis* cells to float in sea water, like dead *Valonia* cells, while those collected on the Pacific Coast sink like living cells of *Valonia*.

The significance of these facts is not clear. It is possible that we are dealing with physiologically distinct but morphologically identical forms. It is also conceivable that the cells cast up on the beaches and studied by Osterhout and Dorcas[1] and by Blinks[4, 6] are, while apparently still living, nevertheless abnormal or possibly moribund.

Perhaps the most satisfactory explanation is that we are dealing with but one species, but that the lower temperature of the Pacific Ocean (15° C. in July when the collections were made) as compared with the Bermuda waters (summer temperatures not far from 25°) may result in a decreased permeability to electrolytes.

That permeability of plant cells to water is decreased by lowering the temperature over this range was shown by van Rysselberghe,[9] and quantitative determinations on *Arbacia* eggs by McCutcheon and Lucke[10] show that their permeability to water is about halved by change of temperature from 24° to 15°. Osterhout[11] assigns a somewhat smaller temperature coefficient ($Q_{10} = 1.33$) to the permeability of *Laminaria* thallus to electrolytes.

Decrease in permeability could, on the basis of the theory proposed by the writer,[7] result in an increasingly preponderant intake of potassium as compared to sodium and in that way cause the observed difference in the cell sap. This presupposes that the permeability to sodium is at the higher temperature already quite high, so that the penetrabilities at the higher and lower temperatures would correspond roughly to positions C and B respectively in Fig. 5 of that paper.

Summary: Normal cells of *Halicystis ovalis* (Lyng.) Areschoug were collected on the Pacific coast from their usual habitat, and the potassium, sodium, and chloride contents of the sap determined.

In contrast to similar analyses reported for drifted specimens of

[9] Van Rysselberghe, Fr., *Acad. Roy. Belg., Bull. Cl. Sciences*, 1901, 173.

[10] McCutcheon, M., and Lucke, B., *J. Gen. Physiol.*, 1927, x, 659.

[11] Osterhout, W. J. V., *Bot. Gaz.*, 1917, lxiii, 317.

the same species collected at Bermuda these cells were found to resemble those of all species of *Valonia* so far studied in exercising selective accumulation of potassium, and resisting the entry of sodium.

4788

Growth and Bone Changes in Rats Injected With Alkaline Anterior Pituitary Extracts.*

MILTON B. HANDELSMAN AND ERNEST F. GORDON.
(Introduced by E. M. K. Geiling.)

From the Department of Pharmacology, School of Medicine, Johns Hopkins University.

Some 175 rats, ranging in weight from 30 to 350 gm. were employed in testing the potency of alkaline anterior pituitary lobe extracts over short periods of time. The method of preparing the extract as described by Evans and Simpson[1] was adopted. In rats weighing between 30 and 100 gm. the presence of the growth hormone could not be detected. However, in animals weighing from 175 to 300 gm., growth stimulating powers were observed. With animals 200 to 225 gm. in weight, after 12 days of injection (the dose being 1 cc. daily intraperitoneally) the experimental rats gained at an average of 2.1 gm. *per diem* over the control rats. After 14 days of treatment with 1 cc. doses intraperitoneally daily, injected animals weighing between 250 and 300 gm. gained remarkably in weight, averaging 2.7 gm. daily over the control animals.

Periosteal bone growth studies of rats were made by including madder in the diet. Madder was shown by Kölliker to be deposited only in zones of osteoblastic activity, staining newly growing bone red. The skulls and mandibles were used as test bones. In young animals periosteal activity was so great that after feeding madder for 2 weeks the bones were diffusely stained pink and made comparative studies impossible. However, in rats over 175 gm., periosteal bone formation was found to be limited to discrete zones of ossification when madder was fed throughout the experiment. The

* After the completion of this work there appeared a communication in this journal by Evans and his associates, Vol. xxvii, Nov., 1929, employing rats weighing between 250-270 gm. His growth results are in accord with ours of the same group.

[1] Evans, H. M., and Simpson, M. E., *J. Am. Med. Assn.*, 1928, xci, 1337.

older the animal, the smaller and more discrete became the ossification zones and the less intensely did they stain. Rats which had received the alkaline extract for 2 weeks showed in the same areas as in the controls a greater activity in periosteal bone formation evidenced by a laying down of more madder. The bones of the freshly autopsied animals appeared redder in contradistinction to the lighter pink color in those of the control animals. Furthermore, the ossification centers in several of the bones had increased in size as demonstrated by a greater area of coloration. The reaction of the bones to anterior lobe administration consisted of an intensification of the activity of the normal periosteal ossification zones. There was no evidence of abnormal zones being created or stimulated.

On comparing the weight and bone changes in the same animals, it was observed that the amount of madder deposited was roughly parallel to the weight increments, that is, those individuals that gained most weight after anterior lobe administration showed the most intensely stained bones and *vice versa*.

4789

Parthenogenetic Development of Eggs in the Ovary of the Guinea Pig.

LEO LOEB.

From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

In 1905 I described,[1] in the ovary of the guinea pig, unusual structures which at first I interpreted as peculiar types of follicular atresia. Further experience, however, convinced me that these structures originated from parthenogenetically developing ova.[2] Since then, long continued study of the ovary of the guinea pig and its various structures has strengthened my conviction that my interpretation was correct.[3] However, a paper by Kampmeier[4] recently expressed the opinion that the structures described by me are not

[1] Loeb, Leo, *Arch. f. mikrosc. Anatomie u. Entwicklungsgesch.*, 1905, lxv, 3.

[2] Loeb, Leo, *Z. f. Krebsforchung*, 1912, xi, 1. *Arch. f. Entwicklgsmech*, 1911, xxxii, 662.

[3] Loeb, Leo, *Science*, 1923, lviii, 35.

[4] Kampmeier, Otto F., *Am. J. Anat.*, 1929, xliii, 45.

embryonic in character but represent abnormal *corpora lutea*.

In view of the great interest in this question and because a fuller publication, for which I had begun to prepare several years ago, has to be delayed, and to prevent the acceptance of erroneous interpretations, I here state briefly the principal reasons which led me to the conclusion (a) that we have to deal in the ovarian structures with embryonic formations and (b) that these embryonic structures owe their origin to parthenogenesis.

(a) The conclusion that we have to deal with embryonic structures is based on the following facts: (1) in 2 animals I observed, within ovaries, early embryos corresponding approximately to the neurula, and in a third case, remains of such structures. One should expect that in the circumscribed area of an ovarian follicle the embryonic development would be very defective; yet, in one case the compression exerted by the rigid wall of the follicle led to only very slight abnormalities, while they were somewhat greater in the second animal. Not only embryonic structures like a neural tube could be readily recognized but, also, the trophoblast was well developed. In more than 30 guinea pigs, we found in the ovary structures closely corresponding to the fetal placenta. These formations are oval or round and the central cavity is lined by an inner layer of cuboidal cells and an outer layer of giant cells and plasmodia; from here chorionic wandering cells penetrate into the surrounding ovarian tissue. They have the tendency to follow the course of the blood vessels and even to penetrate through their wall. This characteristic behavior and the relatively slight resistance offered by such capillaries at the time of oestrus, when there is marked hyperemia, may lead to extensive hemorrhages into the ovary. These bodies are almost identical in character with structures found in the normal embryonal placenta of the guinea pig and with structures found in a case of early extra-uterine pregnancy which I produced experimentally in this species. Moreover, within a single placental body in the ovary, I found combinations of these structures which represent rudimentary trophoblast, and others which represent typical well developed trophoblast of the guinea pig placenta. Another circumstance which gives additional support to my interpretation is the fact that in the ovaries of the same animal we may find in various places multiple new formations representing different types of embryonic structures, namely, in one place a neurula, in another place typical trophoblast with hemorrhage, and in a third place a well developed cystic placental structure. Thus, in a number of cases

multiple structures were found in the 2 ovaries of the same guinea pig.

These facts leave no doubt as to the correctness of our interpretation regarding the character of these bodies. There is certainly not the slightest similarity between these structures and normal or abnormal *corpora lutea;* I have studied the *corpus luteum* of the guinea pig under a great variety of conditions and the various stages in the development of the *corpus luteum* of the guinea pig.[5] Nowhere did I find the slightest resemblance of these embryonal structures to *corpus luteum.* This was also evident in comparing sections of the *corpora lutea* which Dr. Kampmeier found in the dog and which he kindly sent me, with the structures which I found in the guinea pig.

(b) Our conclusion that these bodies owe their origin to parthenogenetic development of eggs and not to fertilized ova, is based on the following facts: (1) In a number of cases the embryonic structures were found in young guinea pigs which had not yet ovulated and were thus sexually immature at the time of examination. This fact alone seems to dispose of the view that we had to deal with fertilization of ova within the ovary. (2) These structures occurred in guinea pigs which had been kept separate from males. (3) They were found in animals during the latter part of pregnancy, and during pregnancy ovulation does not occur. Inasmuch as these embryonal bodies are destroyed through ingrowth of connective tissue after some time, it is improbable that they persist in such a perfect condition for as long a period as 2 months. (4) It is difficult to conceive of a mechanism by which an ovarian fertilization could take place. We would have to assume that following an ovulation, spermatozoa passed through the tube into the peritoneum, that they remained there at least for about 14-16 days and then, at the time of the next ovulation, fertilized the ova. It is very improbable that such a process could occur; however, whether or not this is possible is subject to experimental tests which we intend to make in the near future.

As to the frequency with which these formations occur, all we can state is that they are not exceptional. However, I do not think it of great importance at the present time to make an exact statement as to the frequency of their occurrence. They are not a peculiarity of guinea pigs at any particular locality; they have been found by me in Montreal, Philadelphia and St. Louis. Yet, it is possible that interferences which we applied to produce these bodies

[5] Loeb, Leo, *Anat. Anzeiger,* 1906, xxviii, 102.

experimentally, increased the frequency of their occurrence. In the last few years we have found only one embryonal structure in the ovary of a guinea pig and this was in an animal which had been hysterectomized several weeks previously by Dr. R. J. Crossen in our laboratory. The fact that no intermediate stages between the early irregular segmentation of the ovum and the fully developed embryonal structures have been encountered so far, can easily be understood if we consider the rapidity with which the developing ovum passes through the early stages of development, it then stops when a certain critical stage has been reached at the period of the formation of neurula or embryonal placenta and trophoblast.

4790

A Reversible Experimental Uremia.

B. O. RAULSTON. (Introduced by T. Addis.)

From the Department of Medicine, Stanford University Medical School.

If the *vena cava* in ♀ albino rats is ligated and cut immediately above the entry of the renal veins there is a temporary cessation of renal function and the blood urea rises to very high levels. By the end of the second day a urine is voided which looks like water. It contains a high concentration of protein and many renal failure casts. Ninety day old rats were used. They were taken from the usual stock diet for the colony, operated upon and given only distilled water after the operation. Groups of 8 or 10 were sacrificed at intervals of from one to 6 days after the operation. The average concentration of blood urea, mgm. per 100 cc., on these various days was as follows: On the first day 268, on the second 413, on the third 278, on the fourth 63, on the fifth 55, and on the sixth 31. These rats excreted normally from 2 to 4 mgm. of protein in the urine in 24 hours but during the first and second days following the operation they excreted from 40 to 55 mgm. per 24 hours. Of the 57 rats used in this part of the work 2 died, giving a mortality of 3.5%. As controls similar animals had the same operative procedure carried out except that the *vena cava* was not ligated or cut, or it was ligated and cut immediately below the entry of the renal veins. The highest concentration of blood urea found in these animals was 34 mgm. per 100 cc., there was little or no increase in the amount of protein excreted, and none of them died.

This form of experimental uremia on account of its reversibility and the relative simplicity of its causation, is particularly adapted for the quantitative analysis of certain aspects of the general problem of uremia.

4791

The Crystals of the Follicular Ovarian Hormone.

EDWARD A. DOISY, SIDNEY THAYER AND CLEMENT D. VELER.

From the Laboratories of Biological Chemistry, St. Louis University School of Medicine, St. Louis, Missouri.

The first preparation of crystalline ovarian hormone[1] was obtained from a combination of 2 acidic aqueous solutions containing approximately 7500 rat units of a potency exceeding 1000 units per mgm. The aqueous solution (volume 480 cc.) was extracted with six 150 cc. portions of ethyl ether, the ether distilled and the flask evacuated to remove the last of the solvents. The residue was leached with small volumes of anhydrous ethyl ether, this solution centrifuged, poured into a flask and distilled to dryness. Owing to the danger of ether peroxides 1 cc. of ethyl alcohol was added and distilled using a vacuum to complete the removal of the alcohol. The flask was put in the refrigerator (—10° C.) and crystals began to appear within a short time. The weight of the crystals, which possibly were not absolutely pure, was 2.07 mg. Upon an additional purification the weight diminished to 1.39 mg.

Beginning with this initial crystallization, we have been able to convert all of our preparations into a crystalline form. Many individual preparations have been converted into the pure crystals. Several other products of a different crystalline form isolated during the course of preparation have proved to be inactive upon assay.

One interesting observation regarding the crystalline structure has been made. The pure hormone crystallizes in at least 2 distinct forms of crystals, a phenomenon which is not exceptional but which is encountered quite frequently.

[1] Announcement of this discovery was first made on August 23, 1929, at a scientific meeting of the Thirteenth International Congress. (*Am. J. Phys.*, 1929, xc, 329.) Lantern slides of the 2 crystalline forms were used to illustrate the 2 kinds of crystals, and the evidence regarding the identity of the hormone and the crystals presented.

For want of a more adequate description, we refer to the one form as clusters of needles (A) and to the other as rhombohedral plates (B). In one of our earlier preparations, A separated from a light yellow oil. Upon washing out the oil with cold ethyl alcohol,

PLATE 1. Crystals A of text. 30X.

PLATE 2. Crystals B of text. 416X.

we obtained the A form apparently in a pure condition. Upon recrystallization from dilute ethyl alcohol, the B form was obtained.

As a rule the clusters of needles are obtained when a solution of the pure crystals is freed from solvent by distillation, whereas the plate form appears upon crystallization from hot dilute aqueous acetone or ethyl alcohol or from butyl alcohol. While we have not studied the point in detail, our records indicate that either form may be converted into the other, depending upon the experimental procedure.

4792

Influence of Ingestion of Butter Fat on Body Fat of the White Rat.

HENRY C. ECKSTEIN.

From the Laboratory of Physiological Chemistry, School of Medicine, University of Michigan, Ann Arbor.

In previous communications[1, 2] the writer presented evidence indicating that the ingestion of derivatives of butyric and caproic acids by the white rat exerted an effect on the body fat of such animals quite different from that usually observed when fats are fed. The general effect consists of an alteration of body fat accompanied by an incorporation in that fat of derivatives of the ingested fat. While it was apparent, from the investigations referred to, that the tissue fat of rats had been altered as a result of the ingestion of derivatives of the above mentioned acids, no evidence of the presence of the ingested fats in the fat of the rats could be obtained. The results secured when tricaproin was fed were quite satisfactory, since the rats appeared to be normal and grew at the usual rate. On the other hand those on a diet containing the butyryl radical in the form of sodium butyrate appeared to be abnormal throughout the whole experimental period.

This communication is a report of experiments in which the butyryl radical was incorporated in the diet in another manner, namely by feeding considerable amounts of butter fat to the rats. The diet fed consisted of the following mixture: vegex 2%, salt mixture 5%, casein 20%, butter fat 30%, and cornstarch 43%. Each 100 gm. of food contained non-saponifiable matter from 7 gm.

[1] Eckstein, H. C., *J. Biol. Chem.*, 1929, lxxxi, 613.

[2] Eckstein, H. C., *J. Biol. Chem.*, 1929, lxxxiv, 353.

of cod liver oil. As in previous experiments the animals were kept on the diet for 8 weeks. At the end of that time they were sacrificed by exposure to illuminating gas. The total lipids were then extracted in a manner previously described.[1] Throughout the whole experimental period no evidence of any abnormality was observed.

TABLE I.

*Influence of the Ingestion of Butter Fat and Tricaproin on the Fatty Substances of White Rats.**

Number of rats on diet	Diet	Weight of Animal†	Total lipids	Iodine Number	Saponification Number	Reichert-Meissl Number	Polensky Number
8	Fat Free	140	13.4	68	194	1.28	0.59
7	Tricaproin	159	14.7	59	191	1.28	0.62
10	Butter Fat	149	11.9	63	193	1.16	0.72

* The results presented in the table are the averages for each group.
† Less gastro-intestinal tract.

In Table I are presented the results, together with a summary of data previously obtained. It is quite clear that the amount of fatty acids of low molecular weight is no greater in the fatty substances isolated from the group ingesting butter fat or tricaproin than in the lipids synthesized by rats from fat free precursors. This follows from the great similarity of the Reichert-Meissl and Polensky numbers of the 3 types of lipids. While they are unquestionably alike in this respect they differ somewhat with regard to their degree of unsaturation. The differences between the iodine numbers of the 3 types are not marked, but these are, in the opinion of the writer, true variations and not merely due to the limitations of the method employed. Similar differences were likewise found between the iodine numbers of the fatty acids present in the phosphatides isolated from the 3 types of fat. Those secured from the lipids of animals on the fat free diet had an iodine number of 107, as compared with 101 for those obtained from the lipids secured from rats ingesting butter and 97 for those isolated from the lipids extracted from those on the tricaproin diet.

These results are, therefore, in accordance with those previously reported and again suggest that fatty acids of low molecular weight are, to some extent at least, utilized to build up saturated fatty acids of a higher molecular weight. The difference between the iodine numbers of the control group and that fed butter fat are not as great as those noted between the control group and the tricaproin group. This is to be expected since the percentage of fatty acids of low molecular weight is less in the butter fat diet than in the case of the

diet containing tricaproin. For this reason an attempt is now being made to feed a diet containing much more butter fat with the hope that more striking differences can be secured. Until further work is done the writer wishes to withhold any definite conclusion regarding the effect of the ingestion of butter fat on the chemical composition of body fat.

4793

Quantitative Studies on Precipitins.

JOSEPH G. BAIER, JR.* (Introduced by T. C. Nelson.)

From the Department of Zoology, Rutgers University, New Brunswick, N. J.

Since the quantitative precipitin method of Boyden and Baier[1] has proved to be fairly simple, rapid, and reliable, it was thought advisable to investigate further some properties of this reaction itself. The volume of precipitate obtained in the reaction was studied as affected by (1) quantity of antigen, (2) temperature of the reaction mixture, (3) time and rate of centrifugation, and (4) length of incubation.

Only simple protein antigens have been used in this study (crystalline egg albumin).[2] All protein concentrations have been found by making modified Kjeldahls[3] on samples. The antisera were obtained from rabbits by intravenous injections of the antigen. They were bled from the heart 10 days after the last injection. Calibrated instruments only were used in making all dilutions of antigen and antiserum in buffered saline.

The technique in performing the reaction consisted in preliminary titer determinations (ring test) to ascertain the strength of the antiserum. Following this the quantitative nature of the reaction was studied, using van Allen thrombocytocrits as stated[1] with suitable modifications for particular experiments. The experimental results are shown graphically.

The quantitative technique employed has verified some of the conclusions of earlier workers with regard to antigen-antibody

* The writer desires to thank Dr. A. A. Boyden of this Department for his guidance and advice both in the experimental work and in the writing of this paper.

[1] Boyden, A. A., and Baier, J. G., *J. Immunol.*, 1929, xvii, 29.

[2] Hopkins and Pinkus, *Physiol.*, 1898, xxiii, 130.

[3] Folin, O., and Wright, L. E., *J. Biol. Chem.*, 1919, xxxviii, 461.

equilibrium. Fleishman and Michaelis[4] recognized that the formation of precipitate increases at first with addition of precipitinogen, then decreases and with a certain excess of the antigen approaches zero. Zinsser[5] also noticed the solvent action of excess of antigen. Opie[6] has drawn the same conclusions using crystalline egg albumin as antigen.

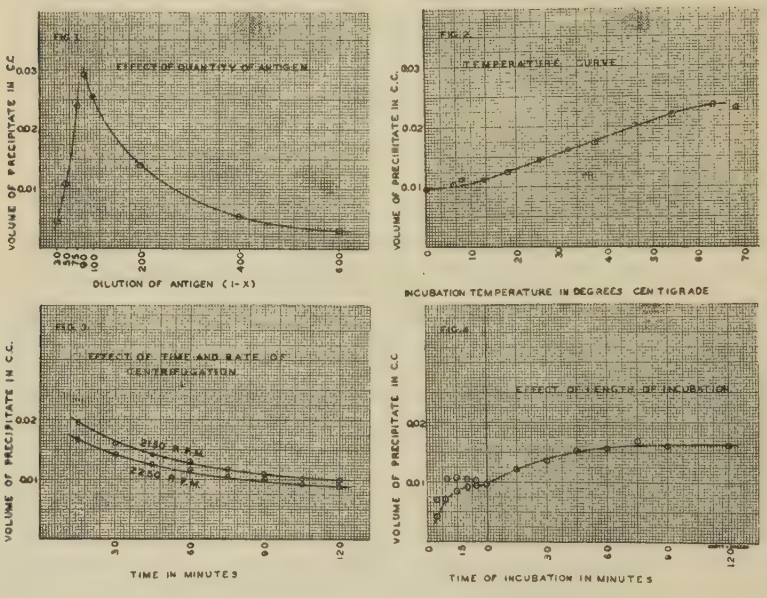

Figure 1 shows the definite effect of antigen dilution on precipitate volume. The antiserum was constant (0.5 cc. of a 1:5 dilution) for all readings, while the antigen was diluted as shown using 0.5 cc. amounts. The curve rises and falls sharply in the region of the maximum precipitate, so sharply in fact that the maximum point can scarcely be found with certainty with the error involved in the readings.

Figure 2 shows, for the first time, the effect of the temperature of incubation on the volume of precipitate. The antigen and antiserum were constant for all readings (0.5 cc. of a 1:150 dilution of antigen and 0.5 cc. of a 1:5 dilution of antiserum). Surprisingly enough the greater part of the curve is a straight line. The point 37.5° C. is in no sense normal for the reaction. Slavish use of

[4] Fleishman and Michaelis, *Biol. Z.*, 1907, iii, 425.

[5] Zinsser, H., "Infection and Resistance," The Macmillan Co., 1914.

[6] Opie, E. L., *Immunol.*, 1923, viii, 1.

this temperature in precipitin work is apparently unjustified as a higher temperature may prove more practicable.

Figure 3 shows the volume of precipitate as influenced by time and rate of centrifugation. The antigen and antiserum were diluted as in Figure 2. The curve is self-explanatory. The greater the rate of centrifugation in similar centrifuges the greater the packing of the precipitate and the less the absolute volume. Obviously, for absolutely comparable results equal centrifugal forces must be employed.

Figure 4 shows the effect of time of incubation on precipitate formation. The antigen and antiserum were diluted as in Figure 2. In the routine technique employed all tubes are centrifuged for 30 minutes after the incubation period. The time of centrifuging, however, is really a part of the incubation period. To get data regarding the volume of precipitate with less than 30 minutes' total incubation it was necessary to centrifuge for less than 30 minutes and calculate the volume for 30 minutes' centrifugation from previous data. Thus the portion of the curve to the left of the Y axis has been indirectly obtained from the points shown above the curve, while that to the right shows the volumes after varying times of incubation plus 30 minutes' centrifuging. The fact that in earlier stages of the reaction a certain rate of precipitate formation is just balanced by the rate of deposition in the thrombocytocrits brings with it a possibility of further studies throwing light on the nature of the reaction itself.

The quantitative data obtained were studied statistically. The probable errors were no larger than those reported in the paper of Boyden and Baier already cited. This means that the results obtained are fairly consistent.

Summary and Conclusions: 1. The quantitative technique described by Boyden and Baier has been successfully employed in a study of the precipitin reaction. 2. The relation of antigen to antibody has been shown quantitatively, using simple protein antigens, to confirm the results of others (Fleishman and Michaelis, Dean and Webb, Opie, Zinsser). 3. The volume of precipitate obtained in one hour's incubation was directly proportional to the incubation temperature. 4. An incubation of one hour at 37.5° C. gives very nearly the maximum precipitate. An incubation of one and one-half hours at the same temperature gives a practically complete precipitation. 5. The rate of centrifugation affects the volume of precipitate formed during a given time and must be considered wherever comparable results are desired.

4794

Physiological Conditions of Ameba Proteus at Varying Hydrogen Ion Concentrations.

SISTER M. ALOYSE ELLINGSON. (Introduced by John Auer.)

From the Department of Biology, St. Louis University, and Webster College.

An effort was made to determine observationally the various interrelationships of physiological manifestations of ameba at varying high pH concentrations in celery infusions. All the cultures were kept under the same temperature and light conditions, the temperatures varying between 21 and 25°C. The same temperature and light conditions prevailed throughout the period of observation. Efforts were made to maintain uniform concentration by the use of like containers and by the addition of 5 cc. of distilled water and infusion every three or four days to each of the cultures. All detailed miscroscopic observations were made with Zeiss Apchromat Lens 3 mm. n.a. 1.4.

In determining pH, the colorimetric drop method of LaMotte with Clark and Lubs' standards was employed.

Quantitative studies on population density at different pH were attempted for the first time. The limits of pH tolerance as stated by Hopkins[1] were extended. 15 of the 22 cultures had a maximum population density at pH 7.8-8.4. A maximum population density occurred in one culture at pH 8.4. Only cultures in which 10 adult ameba in 0.05 cc. of medium occurred were used in the averages. The average density at different pH was as follows:

pH	Average Number of Ameba
7.6	23.41
7.8	15.95
8.0	31.21
8.2	64.21
8.3	103.32

Hopkins has reported a relationship between pH and size, decrease in size occurring at pH 7.8 and higher. In our cultures maximum average size occurred at pH 8.0 and 8.2 (330-405 microns). Larger sizes (600 microns frequently and occasionally 700-900 microns) were found under these alkaline conditions when food supply was abundant.

[1] Hopkins, D. W., *J. Morph. and Physiol.*, 1928, xlv, 1:118.

The average rate of movement of A. proteus from reports of other workers is 0.1400 mm. per minute. Edwards[2] found a maximum of 0.2880 mm. per minute. In our cultures movements between 0.2-0.4 mm. were found in 50.5% of 2,300 records. It should be noted that 45.2% of these lay between velocities of 0.3-0.4 mm. per minute, thus giving the highest rate thus far recorded for A. proteus. 52.5% of 400 readings at pH 8.0 and 53.3% of 390 readings at pH 8.2 gave these high rates of locomotion.

The physiological condition described as "clear" is largely determined by the number of crystals present. At pH 7.4-7.6 the condition described as "dark" was found in 61.85% of 970 observations; at pH 7.8-8.4 only 45.26% of 1,960 observations were found with similar physiological conditions.

Monopodal conditions are reported "almost exclusively" at pH 8.0 and above. In our observations only 45.41% of 2,370 observations were monopodal at pH 8.0, while in 60.34% of 2,774 observations proteus-form occurred at pH 8.2 and 71.35% of 350 readings at pH 8.3 showed a bi- or tripodal condition.

An important by-product of these observations is that crystals have been found on the food mass within the vacuole. During observations on single vacuoles, the crystals were seen merging into the food mass. The evacuation of undigested food material and massed crystals was observed several times, and is also reported for the first time. Further study may settle the question concerning the excretory, secretory or nutritive character of the crystals.

To summarize, excellent and in some cases optimal conditions for various physiological conditions in the ameba have been observed in pH regions 8.0-8.4. The question is still open whether one may conclude that conditions in ameba are relatively independent of pH or whether a series of multi-modal curves with peaks at successive pH levels may possibly be found.

[2] Edwards, J. G., and Fosgrave, H. S., *Johns Hopkins Hosp. Bull.*, 1923, i, 104.

4795

The Prevention of Dental Caries.

R. W. BUNTING, EDNA DELVES AND DOROTHY G. HARD.

From the Laboratories of the School of Dentistry, University of Michigan.

In former contributions on the etiology of dental caries and the means of controlling or preventing the disease[1, 2, 3, 4] the hypothesis has been advanced that dental caries is a distinctly infective disease and that an aciduric microorganism corresponding to the type characteristics of the *B. acidophilus* is the specific etiologic agent. No other factor, thus far ascertained, bears such a constant and intimate relationship to dental caries or harmonizes so fully with the known facts concerning the disease.

For the purpose of studying the effects of certain diets and therapeutic procedures on the activity of *B. acidophilus* in the mouth and the progress of dental caries, a rather comprehensive experiment was conducted during the past year on 3 large groups of children in public schools and orphanages. In 2 orphanages, groups of children were put on a well fortified diet in which sugar was eliminated except as it was used in cooking to make foods palatable. The diet was a varied ration fortified by one quart of milk, green vegetables and fruit for each child daily. These children had no sugar on cereals, in beverages, very little sweetened preserves and pastry, and little or no candy. In addition to the dietary control, Hexylresorcinol (S. T. 37) was used daily as a mouth wash, diluted with 3 parts of water.

In one group of 159 children there were 107, or 66%, in whom there was a marked decrease of *B. acidophilus* in the mouth, and not a single vestige of active caries appeared during the year. Caries was active in only 14 children, or 9%, and in them the disease was limited to but 1 to 3 small cavities per child. In the remaining 25% of the children there were only minute dental defects which were questionable caries. Among the 107 children who had no caries, there were 61 who, at the beginning of the experiment, had open cavities and these cavities remained unfilled throughout the year, but *did not increase in size.*

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[1] Bunting, R. W., and Palmerlee, Faith, *J. Am. Dent. Assn.*, 1925, 381.

[2] Bunting, R. W., *Dental Cosmos*, 1926, 931.

[3] Bunting, R. W., Nickerson, Gail, Hard, Dorothy G., and Crowley, Mary, *Dental Cosmos*, 1928, 1.

[4] Bunting, R. W., Crowley, Mary, Hard, Dorothy G., and Keller, Margaret, *Dental Cosmos*, 1928, 1002.

In a second group of 118 children there were 80 who had no new cavities and 22 who showed slight evidences of dental disease consisting of 1 to 3 small cavities, none were extensive. The remaining 16 children had defects recorded as questionable caries only. Among the 80 children that had no new caries there were 57 who, at the first examination, had open cavities which were not filled during the year and which did not increase in size.

A group of children in a public school were given Hexylresorcinol as a mouth wash twice daily during a period of 9 months' school term, 5 days per week. No attempt was made to alter the regular home diet. At the end of 9 months it was found that among 104 children there were but 24 who had no caries. In 67 children, or 65%, there were marked evidences of active caries, some of which were quite extensive, the clinical picture being vastly different from that which was observed in the 2 other institutions in which both diet and Hexylresorcinol were used.

The data show that in 2 groups of children the activity of oral *B. acidophilus* was markedly reduced and that dental caries was almost completely arrested by the dietary and therapeutic measures employed. As far as we know, this is the first successful experiment in which dental caries has been so completely eliminated from so large a group of children. It appears that of the 2 methods employed, diet was by far the most active inhibitive force, but it will require other carefully controlled experiments to determine just what parts diet and therapeutic remedies severally play in the prevention of dental caries.

4796

Effect of Lutein Feeding on the Oestrus of the Guinea Pig.

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From the Pharmacological and Chemical Research Laboratories, Hynson, Westcott & Dunning, Baltimore, Maryland.

In a previous paper, Macht, Stickels and Seckinger described their experiments with injections of *corpus luteum* extracts on the oestrus cycle of guinea pigs, as studied by the vaginal smear method.[1] It was pointed out that injections of such extracts produced an inhibition of the oestrus and were accompanied by characteristic histo-

[1] Macht, Stickels and Seckinger, *Am. J. Physiol.*, 1929, lxxxviii, 65.

logical findings. Inasmuch as a great deal of the earlier work with ovarian endocrines was done by feeding glandular substance to animals and human beings, it seemed desirable to inquire as to whether or not such feeding experiments in the laboratory would be followed by results similar to those obtained with injections. In the present paper a report is made of the results obtained in a series of feeding experiments on guinea pigs performed in this laboratory for a period of about one year.

The method of experimentation was as follows: A series of guinea pigs was carefully studied to establish the duration of their normal oestrus cycle. This was done by microscopic examination of vaginal smears made daily over long periods of time. After determining the length of the oestrus cycle in each case, the guinea pigs were daily fed from 0.1 to 0.2 gm. of desiccated *corpus luteum* substance suspended in fresh cow's milk. Such a suspension can be easily administered to a guinea pig by means of a glass dropper or pipette. The *corpus luteum* substance used was obtained from the sow. Fresh glands were dried *in vacuo* under slightly diminished pressure at a temperature of from 80° to 85° Fahrenheit. The desiccated gland was then powdered and used, the powdered gland being equivalent by weight to about 20 per cent of the fresh gland. Some experiments were also made with the *corpus luteum* powder which was first extracted in a Sohxlet apparatus with ether. The residue thus obtained gave the same results as the original powder. Other animals were fed similar suspensions or solutions in milk of the follicular hormone, and still others were given milk alone. In addition to the daily feeding of the milk suspensions, all the animals received a plentiful supply of green vegetables. After such feedings for periods varying from four to eight weeks, a study of the vaginal smears obtained during that time was again made. The results obtained are exhibited in the subjoined table. In the large majority of cases, it was found that feeding of *corpus luteum* substance was followed by a definite inhibition of the oestrus, as shown by shortening of the oestrus and prolongation of the dioestral period. In the table are indicated the duration of the normal dioestrus, the kind of gland substance administered, and the resultant duration of the dioestrus after feeding it a given number of weeks. The first 5 experiments show normal controls which make it evident that when no ovarian substance was fed, no change in the oestrus cycle was produced. This held good for *all* the guinea pigs, not only in these 5 experiments but for all the figures given under the heading of "Normal Dioestrus." It will be noted further that a definite in-

hibition followed the feeding of lutein substance but that this effect did not manifest itself very rapidly. The first indications were noted after a feeding period of about 4 weeks and more marked inhibition was observed after administering the *corpus luteum* substance for 6 and 8 weeks. In Experiment 18, an abnormal animal was used, which showed a very frequent oestrus picture so that the dioestral period was of very short duration. In this case, also, feeding of lutein produced a definite inhibition. In Experiments 19 to 23 are shown the results obtained with feeding *corpus luteum* substance for 6 and 8 weeks, respectively, to the same animals.

TABLE I.

Experiment Number	Normal Dioestrus	Feeding	Resultant Dioestrus	Time
	days		days	
1	7	No ovary	8	After 4 weeks
2	9	" "	9	" 4 "
3	8	" "	9	" 4 "
4	10	" "	10	" 4 "
5	12	" "	11	" 4 "
6	8	Lutein	10	" 4 "
7	6	"	11	" 4 "
8	8	"	8	" 4 "
9	10	"	15	" 5 "
10	7	"	10	" 5 "
11	14	"	14	" 5 "
12	11	"	11	" 5 "
13	9	"	12	" 5 "
14	6	"	12	" 6 "
15	8	"	12	" 6 "
16	8	"	12	" 6 "
17	9	"	13	" 6 "
18	2	"	8	" 6 "
19	6	"	15-20	After 6 and 8 weeks
20	9	"	16-35	" 6 " 8 "
21	8	"	16-18	" 6 " 8 "
22	8	"	19-22	" 6 " 8 "
23	8	"	16-19	" 6 " 8 "
24	8	Follicin (C)	3	After 4 weeks
25	9	" (A)	5.5	" 4 "
26	8	" (D)	4.5	" 4 "
27	8	" (B)	2	" 4 "
28	9	" (F)	6	" 4 "
29	12	" (F)	4	" 4 "
30	13	" (E)	14	" 4 "
31	10	" (E)	10	" 4 "
32	8	" (F)	3	" 4 "

In addition to the feeding experiments with *corpus luteum* substance, a number of experiments were made with feeding of the follicular hormone. Some of these preparations were made in this

laboratory. Other preparations of follicular hormone were obtained from the drug market. It was found that administration of the follicular hormone, instead of inhibiting the oestrus, tended to shorten the oestrus cycle.

The results of the research make it quite evident that feeding *corpus luteum* extracts and other ovarian hormones is followed by definite physiological effects, as indicated by a study of the vaginal smears in the guinea pig. When such feeding was discontinued, the animals tended to resume their normal oestrus cycle.

It is interesting to observe that, in a very recent publication, Hisaw noted an experimental relaxation of the pelvic ligaments of the guinea pig by the *corpus lutcum*. Such relaxation was produced also by giving large doses of that hormone by mouth.[2]

The findings of the present writers agree also with other experimental work performed by certain investigators on the effect of feeding ovarian substances to animals. Thus, Löwy and Richter (in 1899) found a definite change in the metabolism produced by feeding ovarian substance to castrated female dogs[3] and very recently, Kochmann, employing the vaginal smear method on mice and rats, described definite changes noted after peroral administration of a number of commercial ovarian products.[4] The evidence furnished by the experiments of Hisaw, Löwy, Richter and Kochmann, as well as by the present authors, is in agreement with numerous therapeutic experiences reported by such eminent gynecologists as Burnam,[5] Leighton,[6] and others. We must, therefore, conclude that the hormones of the ovary, like those of the thyroid gland, belong to those endocrines which are capable of exerting their physiological effects not only after injection but also after oral administration.

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[2] Hisaw, *Physiol. Zool.*, 1929, ii, 59.

[3] Löwy and Richter, *Archiv. f. Anat. u. Physiol.*, 1899, Supp. Vol., 194.

[4] Kochmann, *Archiv. f. exp. Path. u. Pharmakol.*, 1929, cxliii, 57.

[5] Burnam, *J. Am. Med. Assn.*, 1912, lxix, 698.

[6] Leighton, *Trans. Am. Assn. Obstetricians and Gynecologists*, 1915, lxxii, 878.

4797

Relation of the Plasma and Whole Blood CO_2 in Cancer.

FRITZ BISCHOFF, ELSIE HILL AND M. LOUISA LONG.
(Introduced by W. D. Sansum.)

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Van Slyke and Sendroy[1] have worked out a line chart for estimating the factor by which whole blood [CO_2] is multiplied to obtain plasma or serum [CO_2]. This factor is dependent upon the pH, the oxygen capacity and the degree of saturation of the hemoglobin. In the majority of blood analyses presented by these authors, the difference between the observed and calculated value was not over 1 volume per cent. In the values calculated from the data of Peters, Bulger and Eisenman,[2] however, a greater deviation was observed. "The greater variability of these bloods was regarded as due to the fact that they were from a miscellaneous group of hospital patients, many of whom were obviously in very pathological condition."

In studies on the blood chemistry of a series of hopeless cancer patients we determined the data required to calculate the plasma [CO_2] from the whole blood [CO_2]. The plasma [CO_2] was also determined. We are reporting the results because of the abnormal relationship between whole blood and plasma [CO_2] observed in several instances.

The plasma pH was determined by means of the quinhydrone electrode, by a method (slightly modified) recommended by Cullen. The other data were determined by the Van Slyke-Neil manometric apparatus. We have included analyses of the bloods of several noncancerous individuals. The plasma [CO_2] of this group as calculated from the line chart falls well within the error found by Van Slyke and Sendroy. The greatest deviation between the observed and calculated plasma [CO_2] is 1.2 volumes per cent.

The bloods of 11 hopeless cancer patients were studied. Of these the difference between the observed and calculated plasma [CO_2] was abnormally large in 5 cases. In 4 cases the difference was equal to or greater than the 2.5 volume per cent difference found as the maximum difference in the Peters, Bulger, and Eisenman

[1] Van Slyke, D. D., and Sendroy, J., Jr., *J. Biol. Chem.*, 1928, lxxix, 781.

[2] Peters, J. P., Bulger, H. A., and Eisenman, H. J., *J. Biol. Chem.*, 1923, lviii, 773.

TABLE I.—Comparison of the plasma [CO_2] as calculated from the plasma pH, oxygen capacity and hemoglobin unsaturation, by the Van Slyke-Sendroy Line Chart and as experimentally determined for a group of normal and of cancer patients.

Name	Date	pH of plasma at body temp.	Determined		O_2 capacity	O_2 unsaturation	[CO_2]p Calculated from Line Chart	Observed [CO_2]p—Calculated [CO_2]p	
			[CO_2]p in plasma	[CO_2]b in whole blood					
			vol. %	vol. %	vol. %	%			
B. T.	5/14/29	7.43	64.2	51.6	24.0	52	64.2	0.0	Normal
B. M.	3/29/29	7.45	69.7	57.6	22.0	67	70.0	—0.3	"
E. O.	8/24/29	7.50	64.3	54.5	19.4	48	65.5	—1.2	"
W.	8/24/29	7.49	67.2	53.7	22.4	35	67.3	—0.1	"
W. A.	8/ 8/29	7.50	65.5	53.4	20.5	41	65.4	+0.1	"
L.	8/25/29	7.45	65.6	51.4	23.4	32	64.4	+1.2	"
C.	8/ 8/29	7.45	62.0	49.9	21.2	33	61.1	+0.9	Non-malignant tumor
G.	5/25/29	7.52	64.7	54.0	17.4	37	64.2	+0.5	Carcinoma prostate
	5/30/29	7.56	60.1	48.7	17.8	4	59.3	+0.8	" *
	6/21/29	7.51	61.8	51.8	18.7	26	62.6	+0.8	"
C.	6/26/29	7.44	82.2	66.9	18.6	55	79.1	+3.1	Melanoma
	7/ 3/29	7.51	80.0	66.7	16.2	36.5	78.1	+1.9	"
Mc.	10/ 8/29	7.46	68.3	54.0	18.9	10	65.1	+3.2	Carcinoma throat
	11/ 7/29	7.47	68.9	55.7	18.6	60	66.0	+2.9	"
A.	10/17/29	7.52	62.3	54.2	18.5	52	64.8	—2.5	Carcinoma cervix
	11/17/29	7.52	63.4	55.9	15.0	59	64.3	—0.9	"
C.	6/ 7/29	7.49	60.2	54.4	9.9	27	59.4	+0.8	Carcinoma penis
M.	9/10/29	7.58	56.0	50.6	8.6	23	55.1	+0.9	Carcinoma cervix
McK.	8/21/29	7.57	64.2	56.4	12.7	33	64.3	—0.1	Carcinoma† abdomen
Ma.	10/10/29	7.45	61.8	53.4	13.2	31	60.5	+1.3	Carcinoma rectum
T.	10/14/29	7.54	64.1	58.2	11.6	50	65.0	—0.9	Carcinoma stomach, liver
L.	10/26/29	7.53	65.0	54.6	21.7	40	68.0	—3.0	Carcinoma cervix
G.	10/29/29	7.51	60.7	52.1	19.6	29	63.4	—2.7	Carcinoma cervix

* Blood taken during heating experiment, in which body temperature was raised by high frequency electric current. In this experiment the body temperature was raised from 36.6 C. to 37.2 C. The plasma pH rose from 7.48 to 7.56. The plasma [CO_2] fell from 66.0 to 60.1.

† Patient had vomited before the blood was drawn The symbols are those used by Van Slyke and Sendroy.

data. For 3 cases in which the difference was abnormally high, the data were determined again at a later date. In 2 of these, the difference remained abnormal. In the third, the last result was normal. It is interesting to note that this patient received radium and colloidal lead phosphate after the determination of the first data and had shown a marked improvement. The observed value was higher than the calculated in 2 instances of those which showed a maximum deviation. In 3 instances it was lower. These 3 cases all had carcinoma of the cervix. This observation may be entirely fortuitous. Abnormal albumin and globulin contents of blood have been observed in cancer patients.[3] It is probable that an abnormal serum protein content or distribution is responsible for the abnormal results obtained in our series. For 7 of the cancer patients the difference between the calculated and observed plasma [CO_2] was no greater than for the normal group. It should be noted that several of these patients were very anemic.

Summary: In calculating the plasma [CO_2] of blood from cancer patients by the Van Slyke line chart, an error of 2 to 3 volumes per cent may be introduced. Of 11 cases studied, 6 observed values showed a normal agreement with calculated values. Of the 5 values which showed an abnormal deviation, 2 were in one direction and 3 in another.

4798

Egg-Laying in Triturus Viridescens Following Pituitary Transplants.

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From the work of Smith,[1] Smith and Engle,[2] and Engle[3] it is known that anterior pituitary transplants hasten sexual maturity in rats and mice. In females the ovaries contain an excessive number of mature follicles and superovulation occurs. Egg-laying, mating, and subsequent development of the fertilized eggs has also been induced in frogs, in autumn, by Wolf.[4]

[3] Wells, H. G., "Chemical Pathology," W. B. Saunders Company, 1925, 572.

[1] Smith, P. E., *Anat. Rec.*, 1926, xxxii, 221; PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 131.

[2] Smith, P. E., and Engle, E. T., *Am. J. Anat.*, 1927, xl, 159.

[3] Engle, E. T., *Anat. Rec.*, 1928, xxxvii, 275.

[4] Wolf, O., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 692; *Anat. Rec.*, 1929, xliv, 206.

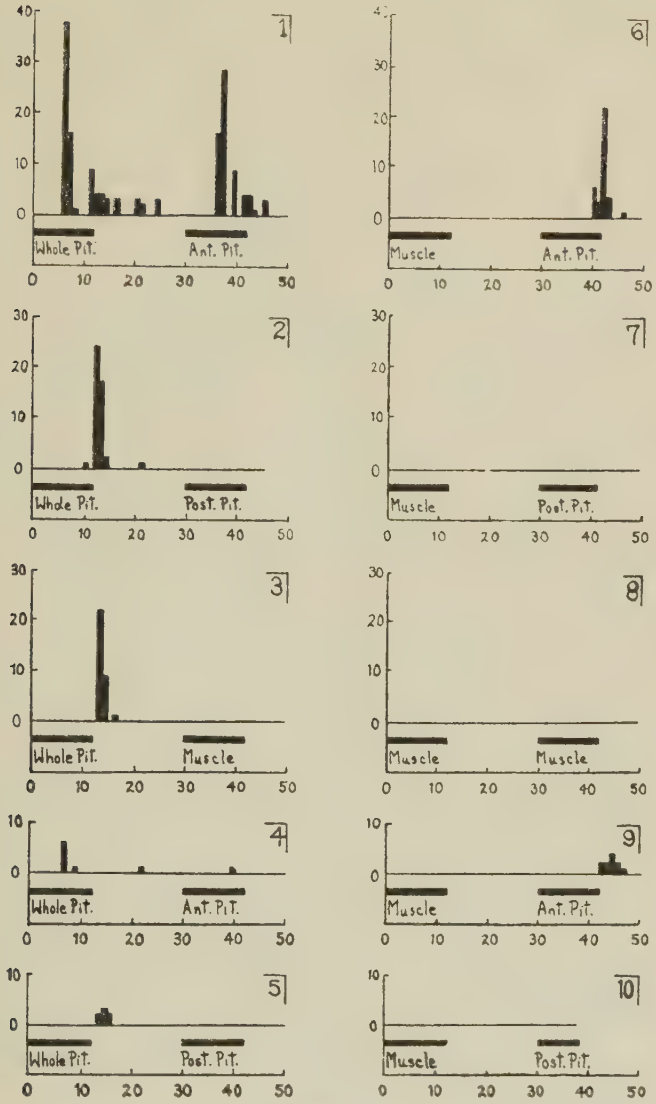
Experiments were undertaken to see if females of urodeles *(Triturus viridescens)* would also deposit eggs out of season if they received transplants of the pituitary gland. In October, a student working with the writer, placed, on alternate days, whole glands in females unilaterally castrated. After 7 transplants one individual laid an egg (October 22nd). Transplants were continued and 15 more eggs were laid up to November 16th when the animal was killed. Six other females also shed eggs at other times after varying numbers of transplants.

Early in December, 5 normal adult females received whole pituitary gland transplants from normal adult males (one transplant daily for 12 days). Five others (controls) were engrafted with small bits of muscle. All grafts were implanted either intramuscularly or intraperitoneally. Animals receiving pituitary glands laid eggs during (Numbers 1, 2, 4) or after (Numbers 3, 5) the grafting (Graph 1). Numbers 1, 2 and 3 laid 86, 45 and 32 eggs respectively over a number of days while Numbers 4 and 5 laid 8 and 7 respectively. The last 2 animals were small as compared with the first 3 and were selected purposely for comparing with the large specimens which from external examination very evidently contained sizable ovaries. None of the animals (Numbers 6-10) which received muscle laid any eggs.

After the egg-laying in the pituitary-engrafted animals ceased, another series of transplantations was begun (January 7th) using the same animals. Numbers 1 and 4 (previously engrafted with whole gland) and Numbers 6 and 9 (previously engrafted with muscle) received anterior lobe while Numbers 2 and 5 (whole gland group) and 7 and 10 (muscle group) received posterior lobe. Number 3 (whole gland group) and Number 8 (muscle group) received muscle. In each animal into which anterior lobe was transplanted, egg-laying occurred (Numbers 1, 4, 6, 9). Again Numbers 4 and 9, the small animals, laid few eggs (1 and 11 respectively) while Numbers 1 and 6 laid 71 and 36 respectively. No animal receiving either posterior lobe or muscle deposited eggs. Another control series which was engrafted daily with a pair of thyroid glands failed to lay eggs.

While these experiments were under way a paper[5] appeared reporting induction of egg-laying in *Eurycea bislineata* by anterior pituitary transplants. The data in the present account therefore add another instance of egg-laying induced in urodeles by pituitary

[5] Noble, G. K., and Richards, L. B., *Am. Mus. Novitates*, 1930, Jan. 9, No. 396.



GRAPH 1.

Effects of pituitary and muscle transplants on egg-laying in *Triturus viridescens*. On ordinates are given numbers of eggs laid; on abscissae, number of days experiment continued.

grafts. These experiments seem to indicate that either anterior lobe or whole gland (anterior lobe being the active factor) may be employed. Whole gland is somewhat easier to use since no time is sacrificed for the separation of the anterior and posterior lobes.

4799

Failure to Confirm Rosenow's Work on Encephalitis in its Relation to Green Streptococcus.*

EARL B. MCKINLEY.

From the School of Tropical Medicine of the University of Porto Rico, under the auspices of Columbia University, San Juan, P. R.

The writer visited the laboratory of Dr. E. C. Rosenow in Rochester, Minnesota, in June, 1929, to observe the work of this investigator on epidemic encephalitis and poliomyelitis. Rosenow very kindly demonstrated to me his methods and technique with material taken from cases of epidemic encephalitis which had come to the Mayo clinic. The writer frankly expressed his skepticism of the rôle of the green streptococcus in epidemic encephalitis and the rather large group of diseases which Rosenow regards as allied conditions. (It should be recalled that Rosenow regards a green streptococcus as etiologically related to a group of 12 or more disease conditions among which are epidemic encephalitis, poliomyelitis, spasmodic torticolis, epidemic hiccup, ulcerative colitis, gastric ulcer, arthritis deformans, rheumatic fever, epidemic influenza, infectious arrythmia, chorea, pulmonary embolism, etc.).

Rosenow was able to demonstrate to me that one could take swabs from the tonsils and nasopharynx of encephalitis cases, wash these swabs in a small quantity of gelatin-Locke's solution, inject healthy rabbits with 0.1 cc. to 0.2 cc. of the unfiltered suspension intracerebrally, produce a meningo-encephalitis in most of the animals so injected and recover *only* a green producing diplostreptococcus from the brains of the dead rabbits. Likewise cultures from the swabs in glucose-brain-broth media revealed green producing streptococci when plated out on blood agar. Similar green producing streptococci were found upon direct inoculation of blood plates with the infected swabs. Gram-positive diplostreptococci were demonstrated in smears from the surface of the brains of animals dying (usually in 24 to 48 hours) following injection, as well as from smears prepared with pipettings from the brain substance.

The writer felt that Rosenow's demonstration was valid, his technique unimpeachable, that each detail of the demonstration should be accepted without question. The interpretation of this

* This work has been supported by the Matheson Encephalitis Commission. For complete references to literature see "Epidemic Encephalitis" Report of a Survey by the Matheson Commission, Columbia University Press, New York, 1929.

work, however, is another matter, and one is not readily convinced that such a demonstration proves anything regarding the relation of the green streptococcus to encephalitis, poliomyelitis, etc. Since most investigators have failed to confirm Rosenow's results and since few, if any, have up to the present used the precise methods of Rosenow we thought, in fairness to Rosenow, that his methods, as employed at the present time, should be applied to the problem in an effort to confirm his findings.

In Porto Rico the bacterial flora of the upper respiratory tract is somewhat different than in the North. Nearly all healthy individuals harbor the green streptococcus in the tonsils and naso-pharynx. We thought it would be of interest to study a series of *normal healthy individuals* here employing the precise technique of Rosenow as he uses with encephalitis and poliomyelitis cases in Rochester. Twenty-five of the personnel of this institution have been so studied. The results are briefly as follows:

1. The precise technique of Rosenow has been used throughout.

2. Swabs were taken from the tonsil and from the naso-pharynx. These swabs were washed off in gelatin-Locke's solution (2 cc.) and 0.2 cc. of this mixture (containing everything from the swab) was injected intracerebrally into rabbits.

3. Fifty rabbits were injected in the above manner. Two rabbits for each normal subject. Forty rabbits died with typical symptoms as produced by Rosenow with material from encephalitis cases. In other words, 80% of the animals died. Rosenow obtained 74% deaths in his series with material taken from the tonsils and naso-pharynx of encephalitis cases; 40% with material taken from contacts with these cases and only 23% with material taken from normal people. Our animals died as follows:

15	rabbits	died in.....	1 day
13	"	" "	2 days
7	"	" "	3 "
2	"	" "	4 "
2	"	" "	5 "
1	animal	died in.....	10 "

4. Green producing streptococci were cultured from the tonsils in all of the 25 subjects. Green streptococci were cultured from the naso-pharynx in all of the 25 normal subjects. In both tonsil and naso-pharynx cultures the glucose-brain-broth in long tubes, as advocated by Rosenow, were employed. In fact every step throughout these experiments was exactly the same as demonstrated to us by Rosenow.

5. Green streptococci were recovered from the brains of 35 out of the 40 rabbits which died. In two instances a hemolytic staphylococcus was cultivated along with the green streptococci. In one case a non-hemolytic staphylococcus alone was cultured. Two rabbits died in which no organisms could be demonstrated. Hemorrhages in the brain were found, probably due to mechanical injury of the injection.

6. Two animals out of a total of 50 injected developed chronic lesions.

7. In 31 instances direct smears from the surface of the base of the brain revealed Gram-positive diplococci or definite streptococci. (It should be mentioned that Rosenow also finds for the most part diplococci and not chains.) Nine animals were negative.

8. Positive blood cultures from the heart blood were obtained in 18 animals. Direct smear from the heart blood was positive in only 2 animals.

9. Streptococci or diplococci were found in smears from the various organs at autopsy as follows:

Organ	*No. instances positive*
Kidney	5
Liver	5
Adrenal	4
Spleen	4
Lung	5

10. Fermentation reactions:

Rosenow Strains (47)		*McKinley Strains* (42)
47	Glucose	40
22	Lactose	36
38	Maltose	39
31	Saccharose	38
21	Raffinose	34
10	Salicin	15
13	Inulin	13
0	Mannite	1 (?) (Definitely negative third test)

11. Agglutination reactions: Rosenow's anti-encephalitis serum and anti-poliomyelitis serum were used against our strains. The results were as follows: (These tests were run on 26 strains isolated directly from the tonsil or naso-pharynx of the healthy subjects and 25 additional strains isolated from the brains of rabbits which died

following injection.) 51 strains tested in all. All agglutination tests were carefully controlled.

	Anti-Encephalitis Serum (Rosenow)				Anti-Poliomyelitis Serum (Rosenow)			
No. of strains giving positive agglutination:	1/40	1/100	1/200	1/400	1/40	1/100	1/200	1/400
	48	39	30	25	43	38	28	22

12. Virulence tests with 24 strains chosen at random show these streptococci to be practically non-virulent for mice (intraperitoneal injections—1 cc.) and for guinea pigs in the same dosage. Only three out of 24 mice died and only 7 guinea pigs. This is in accordance with virulence tests run with green streptococci by other workers.

13. Three monkeys injected intracerebrally with 1 cc. of 3 different strains chosen at random remained perfectly well. No doubt strains might have been found which would infect monkeys if sufficient numbers were tested.

14. The picture in histologic sections is one of acute meningoencephalitis the same as demonstrated to me by Rosenow in his laboratory. The meningi are hyperaemic and slightly infiltrated with lymphocytes, leucocytes (polymorphonuclears) and plasma cells. There is perivascular infiltration consisting of leucocytes, lymphocytes and plasma cells, as well as diffuse leucocytic infiltration of the subependymal tissue. Gram-positive diplococci, sometimes in short chains and often phagocytized by leucocytes, are found in the subependymal tissue. We have experienced the same difficulty as Rosenow in always finding the microbe in the brain sections, sometimes searching for a long time, but usually they are found to be present though in most instances they are very few.

15. Rosenow's "soaked-pledget-in-the-nose" method of infection has been found very unsatisfactory. Seven rabbits were treated in this manner with pledgets soaked in streptococci but only 3 succumbed to infection.

16. One case of epidemic encephalitis has been studied thoroughly as a control in this series and from this case a green producing streptococcus has been isolated from the tonsils and nasopharynx. With the same technique as employed in all of the other cases precisely the same results have been obtained. In other words, it is impossible to discern any difference between the results obtained by this method with material from encephalitis from those obtained with material from healthy individuals.

17. We have found it perfectly easy and simple to produce a fulminating meningo-encephalitis in the brains of rabbits by injecting a very small quantity of *B. subtilis* (a known saprophyte) and we attach no more importance to this, in relation to the etiology of epidemic encephalitis, than we do to the green streptococcus. In the light of this control work on healthy subjects we believe we would be as much justified in incriminating the Hay bacillus as we would the green streptococcus as the etiological agent in epidemic encephalitis.

We believe that Rosenow's work proves *only* that one can inject into the brains of rabbits material taken from the tonsils or the naso-pharynx of diseased or *healthy* individuals and most of the animals will die of meningo-encephalitis (74% his highest figure with material from encephalitis cases—80% our figure with material from healthy individuals.) One recovers in most instances *only* a green streptococcus—the other microbes having no effect. (In one case we recovered a non-hemolytic staphylococcus alone and in 2 instances we cultured both a hemolytic staphylococcus and green producing streptococcus from the brains of our rabbits.) This phenomenon may be explained on the basis of greater virulence of the green streptococcus or by the fact that they predominate in the flora at the time the swab is taken. In any case it apparently has no bearing upon the etiology of epidemic encephalitis or poliomyelitis and is entirely a problem of more or less academic nature.

Minnesota Section.

University of Minnesota Medical School, January 29, 1930.

4800

"Para-Agglutination and Para-Heredity."

MARY E. HANSEN. (Introduced by A. T. Henrici.)

From the Department of Bacteriology and Immunology, University of Minnesota.

The term "Para-agglutination" was introduced by Kuhn and Woithe[1] to designate the following phenomenon: Colon bacilli isolated from the feces of a dysentery patient were found to be agglutinated with antidysentery serum at a much higher dilution than strains of colon bacilli obtained from other sources.

The term "Para-heredity" was introduced by Wollman and Wollman[2] to designate the acquisition of agglutinability by anti-typhoid serum, by colon bacilli which had been grown together with typhoid bacilli in broth.

Apparently similar phenomena are the so-called "hybridization" of typhoid and dysentery bacilli reported by Almquist,[3] the acquisition of toxogenicity by non-scarlatinal streptococci when grown in scarlet fever streptococcus filtrates reported by Frobisher and Brown,[4] and the recently described "hybridization of proteins" of Manwaring.[5]

I have performed the following experiments: Colon bacilli were cultivated in broth with typhoid bacilli, in one experiment for 8 generations, in another for 17. From the last generation of each series a number of lactose fermenting colonies were isolated and their agglutinability with anti-typhoid serum was noted. In no case was the organism agglutinated in higher dilution than was the original strain which had not been associated with typhoid bacilli.

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[1] Kuhn and Woithe, *Med. Klinik*, 1909, 1709.

[2] Wollman and Wollman, *Compt. Rend. Soc. de Biol.*, 1926, xciii, 1568.

[3] Almquist, *J. Inf. Dis.*, 1924, xxxv, 341.

[4] Frobisher and Brown, *J. Bact.*, 1927, xiii, 44.

[5] Veblen, B. B., Proc. Soc. Exp. Biol. and Med., 1929, xxvii, 204.

Colon bacilli were isolated from the feces of 4 rabbits and their agglutinability with anti-typhoid serum was noted. Each rabbit was then immunized with 4 inoculations of heated typhoid bacilli, followed by 4 injections of living organisms. Intraperitoneal inoculations were made. After this period colon bacilli were again isolated from their feces and tested with anti-typhoid serum. Neither before nor after the series of injections were colon bacilli found which were agglutinated at titres higher than 1-10 with the typhoid serum used.

Staphylococcus aureus was cultivated with *Serratia marcescens,* and *Bacillus megatherium* was associated with the same chromogen, through 9 generations in broth. After that plates were prepared and numerous pink colonies were isolated. In no case was a red chromogen found having any of the characters of either *S. aureus* or *B. megatherium.*

4801

Elective Localization and Cataphoretic Potential of Streptococci.

EDWARD C. ROSENOW AND LLOYD B. JENSEN,

From the Division of Experimental Bacteriology, The Mayo Foundation, Rochester, Minnesota.

Measurements of cataphoretic potential have been made with the Northrop-Kunitz-Mudd apparatus. All suspensions were washed and measured in tested distilled water at 124 volts, 23° C. Determinations of pH of the suspensions were made colorimetrically. The streptococci were grown in tall columns (12 cm.) of glucose-brain broth, in tubes (1.5x20 cm.), for 18 hours at 35° C. Significant, and perhaps characteristic, potentials of green-producing streptococci have been obtained in a considerable number of different diseases, but in this preliminary paper we wish to report only on those obtained in studies of chronic encephalitis and chronic infectious arthritis, together with controls.

Material was obtained from a series of 18 cases of encephalitis and allied conditions. Streptococci obtained from the nasopharynx and from other foci of infection were subjected to tests of potential directly in suspensions and in cultures in glucose-brain broth. Single colonies of green-producing streptococci, obtained originally from the nasopharynx, from other foci of infection, from the stools, and even from the blood, were cultured in glucose-brain broth and sub-

jected to tests of potential in this state. The potential, in these studies, in nearly all of a large number of tests, was *circa* 8.0 (μ/sec). Similar tests of streptococci from a series of 26 cases of chronic infectious arthritis yielded a potential of *circa* 10.6 (μ/sec) in a high percentage of a large number of experiments.

We have used the methods of intracerebral injection and direct injection into the right knee joint, of intravenous injection in rabbits and intraperitoneal injection in mice, in attempts to correlate elective localizing power of the respective streptococci with their cataphoretic potentials.

The streptococci isolated from the brains of animals in which encephalitis developed following injection of material from cases of encephalitis, or from other sources containing "neutrotropic" streptococci, had a potential, chiefly, of *circa* 8.0 (μ/sec). The inflamed joints of animals in which arthritis developed following injection of material from cases of chronic infectious arthritis, or from other sources containing "arthrotropic" streptococci yielded streptococci of a potential, chiefly, of *circa* 10.6 (μ/sec). The potential of other bacteria from these sources was too variable to be of significance.

Dissociation and loss of elective localizing power, with concomitant changes in potential difference of streptococci frequently resulted when media other than tall columns of glucose-brain broth were used even for primary culture. There was also close parallelism between the loss of localizing power of a strain and the number of subcultures, even in glucose-brain broth, especially if subcultures were made only once a day. The condition of latent life, as in suspensions in glycerin, in sealed blood-agar slant, and in meat infusion, has been found often to maintain specific localizing power and concomitant characteristic potentials.

When a primary culture in glucose-brain broth was of but one mobility in the cataphoretic cell, and a graded dose was injected parenterally into rabbits, only one type of lesion usually resulted. If the cocci were of several mobilities in a given culture, several types of lesions might develop in the test animal.

We have used the methods of Shibley, and of Mellon and Grenquist in detecting reactions between antibodies and bacteria by cataphoresis. Seventy serums of patients, in 6 dilutions, to which autogenous streptococci had been added, were tested. Each test was controlled with pooled normal serum, individual normal serum, heterologous patients' serum, distilled water, and saline menstrua and determinations of pH.

Positive serum potentials were obtained in 9 of 26 serums from patients suffering from spasmodic torticollis, in 7 of 30 serums from patients with encephalitis, in 2 of 6 serums from patients with chronic infectious arthritis, in one of 2 serums from patients with chronic ulcerative colitis, in one of 2 serums from patients with corneal ulcer, and in one of 4 serums from patients with localized gastritis or gastric ulcer. In most instances in which positive serum potentials were obtained, agglutination also was obtained. In one serum from a patient with Parkinson's disease following encephalitis whose condition improved remarkably following removal of foci of infection and prolonged use of an autogenous vaccine and serum, the agglutination was marked for neurotropic streptococci in dilutions as high as 1:1000.

No positive serum potentials were obtained with strains from cases of arthritis or from other sources, when the potential difference (μ/sec) was other than 10.0 to 11.0, usually 10.6 (μ/sec). Likewise when organisms obtained from cases of spasmodic torticollis, multiple sclerosis and encephalitis were used, no positive serum potentials were observed with strains other than those with mobilities 7.8 to 8.4, usually 8.0 (μ/sec). This may account, in part, for many of our negative results. As the work progressed, we succeeded in eliminating some, but not all, of the disturbing factors and positive results were obtained more frequently.

4802

Streptococci in the Lesions of Experimental Poliomyelitis in Monkeys.

EDWARD C. ROSENOW.

From the Division of Experimental Bacteriology, The Mayo Foundation, Rochester, Minnesota.

Thorough search for bacteria has been made in stained sections of the central nervous system of 60 monkeys in which symptoms and lesions of varying degree, typical of poliomyelitis, had devel-

[1] Mellon, R. R., and Grenquist, Ernst, *J. Immunol.*, 1926, xi, 161.

[2] Rosenow, E. C., *J. Am. Med. Assn.*, 1921, lxxvi, 1745.

[3] Rosenow, E. C., *J. Infect. Dis.*, 1924, xxxiv, 329.

[4] Rosenow, E. C., Towne, E. B., and Wheeler, G. W., *J. Am. Med. Assn.*, 1916, lxvii, 1202.

[5] Shibley, G. S., *J. Exp. Med.*, 1924, xl, 453; 1926, xliv, 667.

oped following inoculation of virus. Unmistakable Gram-staining cocci and diplococci of varying size, shape and grouping, were found in or adjacent to lesions in 54 of the 60 monkeys. The lesions in the 6 in which diplococci were not demonstrable in the sections available were relatively slight. Three of these 6 died, 12, 18, and 21 days, respectively, after onset of paralysis; the remaining 3 had mild attacks of poliomyelitis and were despatched by anesthesia, respectively on the tenth, eleventh and fourteenth day after the onset of the disease. Of the 54 monkeys in which diplococci were found, 31 were despatched by anesthesia and 23 died from paralysis.

A similar search was made for bacteria in sections of the central nervous system of 60 monkeys in which there were no active lesions of poliomyelitis. These 60 animals were used as controls; 32 were despatched by anesthesia and 28 died from various causes other than poliomyelitis. In none of the sections from the 60 monkeys used as controls were diplococci demonstrable.

4803

The Determination of Surface Area of Living Children.

EDITH BOYD, RICHARD E. SCAMMON AND DONOVAN LAWRENCE.

From the Institute of Child Welfare and Department of Anatomy, University of Minnesota.

Three major methods for obtaining surface area have been applied to both the living body and the cadaver. The first is the geometric. The second is to cover the body with pieces of paper of known area or with a thin sheet of pliable but non-elastic substance which is later measured with a planimeter, by direct weighing or by weighing pieces of paper of the same area as the coating. The third method is the application of the surface integrator.

A variation of the coating method has been devised and applied to plaster of Paris casts of living children.[1] This use of the intermediate stage of a model permits a check on the reliability of the coating method through *seriatim* measurements.

The casts were obtained as follows: The child, after having his body coated with stearine and his hair covered with a close-fitting silk stocking top, was placed on his back in a thick layer of semisolid,

[1] Boyd, E., and Scammon, R. E., *Anat. Record*, 1927, xxxv, 5.

quick-setting plaster of Paris with his legs apart, arms away from the sides and the fingers spread apart. The child was removed when the plaster was sufficiently set to hold its form. While the half-matrix cooled and hardened, its rough edges were smoothed, and its entire surface greased. Then the child was replaced in it and a thick layer of plaster of Paris spread over the body to the chin. This upper matrix was removed in several segments. To obtain the face, the neck segment was refitted leaving the rest of the body free, the closed eyes covered with vaseline and soft tissue paper, and then thick plaster spread over the face, leaving only the anterior nares uncovered. It is essential to grease the child thoroughly, to remove him before the plaster becomes uncomfortably hot, and to keep him sufficiently interested so that he will remain quiet voluntarily. The

FIG. 1.
A photograph of subject P. L., age 4 years 11 months, lying in the lower matrix of the cast preparatory to the application of the upper matrix.

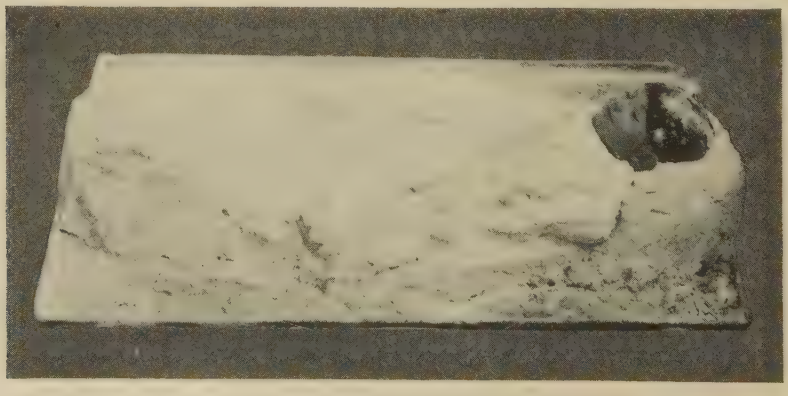

FIG. 2.
A photograph of the same subject at Fig. 1, with the upper matrix *in situ*.

plaster used set in 3 to 15 minutes depending on its consistency when applied. Figures 1 and 2 show 2 steps in the process.

The cast of the child was made by greasing the inner surface of the shell, reinforcing with iron rods, sealing the segments, filling the cavity with liquid plaster and then chiseling the matrix from the resulting cast. Several days are required between each step of the process for drying and hardening of the plaster.

The areas were determined by wrapping the entire cast with overlapping layers of surgical adhesive tape. The ears and fingers were covered separately. The toes were separated by sawing through the indicated divisions and the adhesive pressed into these cuts as far as possible. The edges of the adhesive were colored with crayon, so that any slipping would be apparent. The coating was removed according to the regions of the body described by Du Bois and Du Bois.[2] The adhesive was cut through to the cast with a microtome knife and the lowest layer of adhesive grasped and stripped off. The curved coating of each region was cut into sufficiently to flatten it. These pieces were placed on paper covering sheets of "Kodaloid No. 3"[3] spread on a flat surface. The outlines of the pieces were traced with a microtome knife pressing hard enough to cut through the paper and underlying Kodaloid. The area of each region was determined by weighing the Kodaloid pieces.

The results of repeating this process 2 or 3 times on each cast are given in the accompanying table with the mean relative deviation and total range of relative deviation for the average surface area of each cast.

These give a rough measure of the variability in the coating process but not of the casting. To test the reliability of both procedures, a cast was made of a bowling ball (used for the same purpose by Sawyer, Stone and Du Bois[4]). The areas of both the ball and its model were computed from determinations made with 2 standard physical instruments, the cathetometer and the spherometer, as well as from the adhesive coat.

As shown in Fig. 3, the variability in readings of the surface area for both balls is smallest by the cathetometer, intermediate by the coating method and largest by the spherometer. Thus, the coating method appears to be of the same order of reliability as the methods commonly employed for determining the area of spherical surfaces.

[2] Du Bois, D., and Du Bois, E. F., *Arch. Int. Med.*, 1915, xv, 868.

[3] A celluloid composition used for photographic films. For tests of its accuracy, see Scammon, R. E., and Scott, G. H., *Anat. Record*, 1927, xxxv, 269.

[4] Sawyer, M., Stone, R. H., and Du Bois, E. F., *Arch. Int. Med.*, 1916, xvii, 855.

TABLE I.
Determinations of Surface Area of Living Children.

Subject and sex	Age (yrs. and mo.)	Weight (kg.)	Length (cm.)	Surface area			
				Single readings (sq. cm.)	Means (sq. cm.)	Relative deviations (per cent) Mean	Range
E. H. (f)	3:3	15.63	100.3	6753.6 6891.2 6771.9	6805.6	0.8	2.0
" "	3:4	15.76	100.2	6708.8 6911.0	6809.9	1.5	3.0
" "	3:5	16.20	101.5	6877.5 6945.8 6960.8	6928.0	0.5	1.2
" "	3:6	16.55	102.9	6973.5 6950.1 7013.7	6979.1	0.3	0.9
" "	3:10	16.90	104.8	7039.3 6871.5 6956.9	6955.9	0.8	2.4
R. C. (f)	4:4	15.75	101.7	6565.1 6513.2	6539.2	0.4	0.8
" "	4:9	16.69	104.1	7399.6 7301.8 7361.0	7354.2	0.5	1.3
" "	4:10	16.40	105.2	7406.6 7313.4	7360.0	0.6	1.3
P.L. (m)	4:11	17.20	111.5	7347.1 7436.1 7479.6	7420.9	0.7	1.8

The error in casting is probably best indicated by the comparison of the areas by the cathetometer of both the bowling ball and its model. The latter is 1.5% larger. The ratio of the difference in size of these 2 objects to its probable error is 13.9. This is a significant difference. But the areas of the cast both by the cathetometer and by the coating method are not significantly larger than the area of the bowling ball by the spherometer.

There is apparently a close agreement in the estimates of the area of the cast, as determined by the spherometer, and the area of the bowling ball, as determined by the cathetometer. In this case the larger size of the model (due to the expansion of the plaster) is probably offset by the sinking of the sharp-pointed legs of the spherometer into the softer cast. Moreover, the ratio of probable

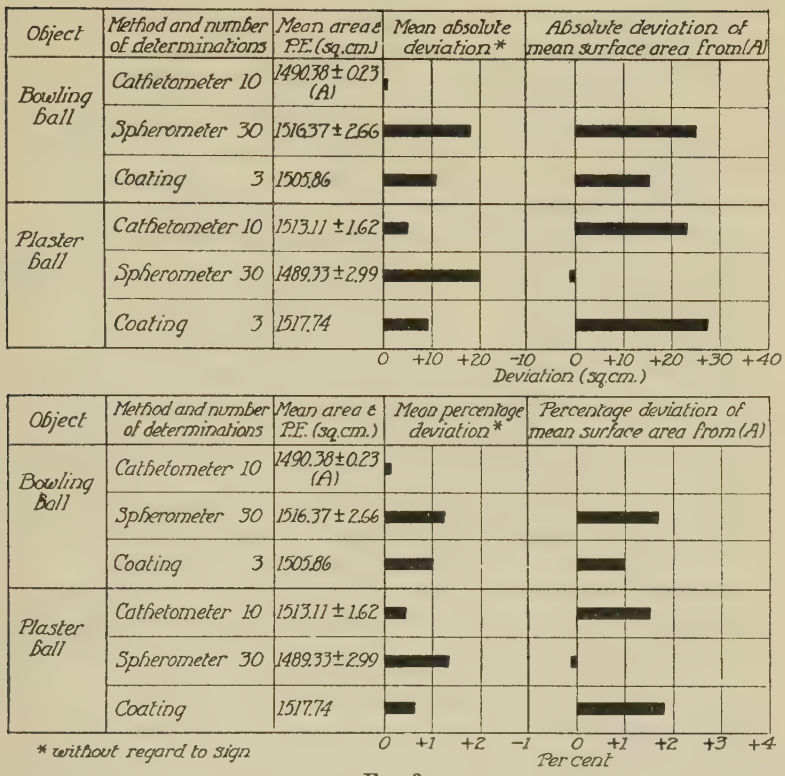

FIG. 3.

A histogram illustrating the variability of the method used on test subjects to determine the precision of the casting and coating method.

error of the difference shows that the area of the plaster ball by the spherometer is significantly less than its area by the cathetometer.

These comparisons indicate that the total error of the casting and coating method is of the order of 2%.

4804

The Relation of Surface Area to Body Weight in Postnatal Life.

EDITH BOYD AND RICHARD E. SCAMMON.

From the Institute of Child Welfare and Department of Anatomy, University of Minnesota.

The 9 determinations of surface area on 3 children from 2 to 5 years reported in the first paper of this series, are not sufficient to warrant the computation of a formula for surface area at this age

level but their relation to the trend of previous determinations by other methods on both living and dead may be determined.

A preliminary survey of the literature furnished 135 actual determinations of the surface areas of normal individuals who ranged from birth to late maturity in age and whose heights and weights were given. The minimal body weight in this series was 2.5 kg. The sources and methods are as follows: Fubini and Ronchi,[1] 1 living adult, geometric method; Meeh,[2] 16 living males, 6 days to 66 years, coating and geometric method, Ssytscheff,[3] 23 males and females, 15 days to 43 years, coating method; Lissauer,[4] 8 male and female cadavera, 28 days to 15 months, coating method; Lassablière,[5] 15 determinations each based on averages of 2 or 3 living children, one day to 24 months, geometric method; Pfaundler[6] (including Kastner), 14 infant cadavera from one-fourth month to 18½ months, coating method; Du Bois *et al*,.[7, 8] 8 living males and females, 12 to 32 years, 1 female cadaver, 21 months, coating method; Wörner,[9] 16 living males and females from 5 to 50 years, coating method; and Frontali,[10] 33 living boys and girls from 24 days to 12 years, integrator method.

The trend of increment of surface area of these cases with body weight is shown in Fig. 1. This trend has been fitted by 2 variations of the method of least squares. The first expression is:

$$S = 1008W^{0.692} \quad (1)$$

where S is surface area in square centimeters and W is weight in kilograms. The constants were fitted by the least squares of the logarithms of surface and weight. Measures of goodness of fit of this expression are shown in the upper panel of Fig. 1.

The second formula is:

$$S = 1070W^{0.675} \quad (2)$$

In this instance the constants of the equation were obtained by assuming a series of exponents, 0.62 to 0.72, that covered the range of powers found in various attempts to fit certain of these data by

[1] Fubini and Ronchi, *Moleschotts Untersuchungen*, Heft 1, xii.
[2] Meeh, K., *Z. f. Biol.*, 1879, xv, 425.
[3] Ssytscheff, A. I., *Diss.*, St. Petersburg, 1902.
[4] Lissauer, W., *Jahrb. f. Kinderheilk.*, 1903, lviii, 392.
[5] Lassablière, P., *Compt. Rend. Soc. Biol.*, 1910, lxviii, 339.
[6] Pfaundler, M., *Z. f. Kinderheilk.*, 1916, xiv, 48.
[7] Du Bois, D., and Du Bois, E. F., *Arch. Int. Med.*, 1915, xv, 868.
[8] Sawyer, M., Stone, R. H., and Du Bois, E. F., *Arch. Int. Med.*, 1916, xvii, 855.
[9] Wörner, H., Z. *f. gesamte Exp. Med.*, 1923, xxxiii, 510.
[10] Frontali, G., *Riv. d. Clin. Pediat.*, 1927, xxv, 241.

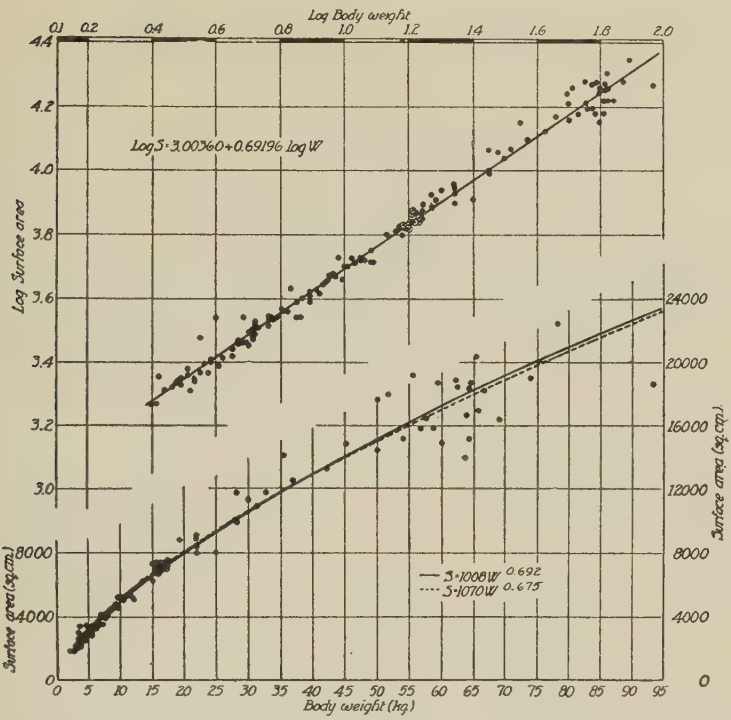

FIG. 1.

Body weight and surface area in postnatal life. Upper graph shows relation of logarithms of surface area to logarithms of body weight. Lower graph shows relation of body surface to body weight. Individual observations represented by solid dots. The 9 observations in the present series represented by circled dots. Analytic expressions indicated by solid lines.

other methods, and selecting the one giving the lowest sum of the squares of the deviations. The details of this method will be given in a subsequent publication.

Since formulae involving both height and weight are commonly considered more reliable for prediction of surface area than those based upon weight alone, the constant according to the Du Bois and Du Bois height-weight formula[11] was solved for these data, giving the expression:

$$S = 76.40W^{0.425}H^{0.725} \quad (3)$$

The mean relative deviation of the first constant is 6.3%. Also the mean relative deviation of the calculated values for this formula from the observed values of surface area is less (6.3%) than that (7.3%) for the original Du Bois formula:

$$S = 71.84W^{0.425}H^{0.725} \quad (4)$$

[11] Du Bois, D., and Du Bois, E. F., *Arch. Int. Med.*, 1916, xvii, 863.

Using Du Bois' principle of a bidimensional formula:

$$S = W^{1/a} \times H^{1/b} \times c$$

when $1/a + 1/b = 2$

we varied 1/a from 0 to 0.667 and obtained

$$S = 394.56W^{0.575}H^{0.275} \quad (5)$$

as the expression with the lowest mean relative deviation for the first or "c" constant, 5.1%. According to Du Bois' criterion (5) is a better fit to these data than (3) and (4).

The percentage deviations of the calculated values from the observed were computed to compare the departure of these determinations from the values obtained by the formulae. The frequency distributions of these percentage deviations and their interquartile ranges for each formula are given in Fig. 2.

The average percentage deviation and the root mean square deviations for each 25 cases in order of magnitude of body weight for

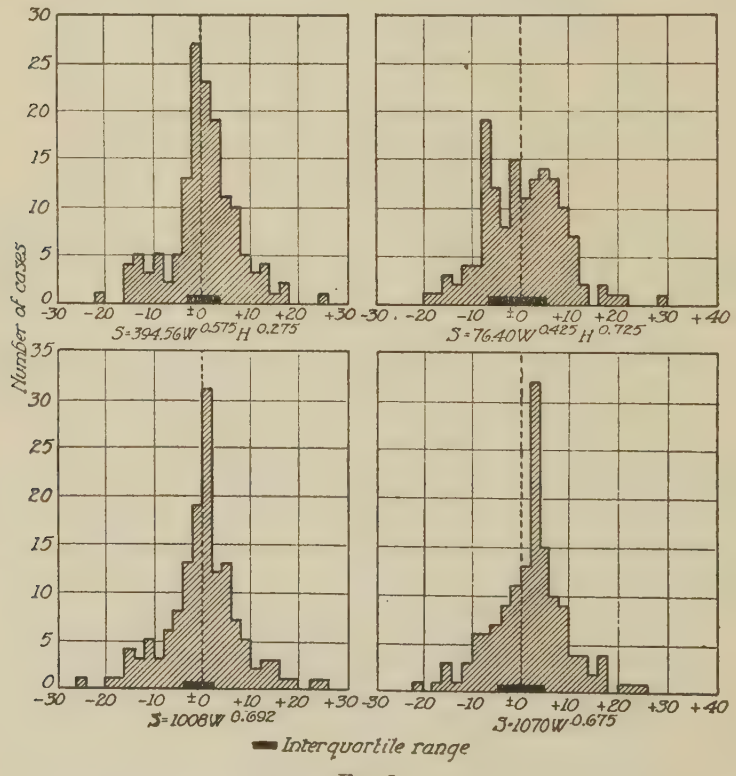

FIG. 2.

Histograms illustrating the distribution of the relative deviations of calculated surface areas of 2 weight and 2 height-weight formulae from the corresponding observed values.

formulae (1), (2), (4) and (5) are given in the table. The weight formulae are scarcely as good fits for the lower weight levels, although all the expressions give prediction values of the same general order of precision except for the last weight group (57.62 to 93.01 kg.). Here the cases are few and certain of the individuals used were "atypical" in body form.

TABLE I.
Deviations of Calculated from Observed Surface Areas for 144 Observations.

Weight intervals (kg.) and number of observations in each interval	Mean relative deviations (%) of formula:				Root mean square deviations (sq. cm.) of formula:			
	(1)	(2)	(4)	(5)	(1)	(2)	(4)	(5)
2.50 to 4.14 (25)	6.7	8.2	7.7	7.1	257	264	273	232
4.45 to 6.28 (25)	3.2	5.3	9.0	3.1	143	188	324	135
6.50 to 11.21 (25)	3.9	5.3	10.0	3.7	226	266	508	227
11.30 to 18.72 (25)	3.1	3.5	3.9	2.9	272	282	371	255
18.75 to 56.91 (25)	7.2	6.6	6.6	6.3	1228	1093	1174	1160
57.62 to 93.01 (19)	9.3	9.0	6.3	7.9	2031	1956	1577	1715
Total range 2.50 to 93.01 (144)	5.4	6.2	7.3	5.0	902	854	806	796

These comparisons seem to justify the following conclusions in so far as the present data* are concerned. Surface area *in the growing period* can be calculated with about equal precision from expressions with constants determined by least squares for weight alone, and from expressions derived by the geometric method of Du Bois and based on both weight and length. In this period the relation of surface to weight closely approximates the expression

$$S = cW^{2/3}$$

regardless of differences in method of determining surface area or whether these methods are applied to living individuals or cadavera.

* Since the calculations were made, 50 additional observations have been found in the literature and these will be included in an analysis of all available data in a final report.

4805

Effect of Forced Exercise on Size of Heart in Normal and Pericardiotomized Dogs.

G. FAHR, O. WANGENSTEEN AND S. SPERLING.

From the Departments of Medicine and Surgery, University of Minnesota.

An effort was made to determine whether or not the pericardium plays any rôle in limiting the size of the heart following severe exercise. The pericardium of 3 dogs was so incised as to remove any restraining influence. Two normal dogs of approximately the same weight and size as the others were used as controls. The operated dogs were observed over a long period of time (6 mo.) during which x-ray plates of their hearts were taken. The mean deviation of the mean value of the silhouette area, as measured over this period of time, and covering many measurements, was $\pm 3\frac{1}{2}$ sq. cm., or about 5% of the total area.

During this period the pericardiotomized dogs and the normal dogs were trained to run on a treadmill. They were forced to run approximately 1 hr. each day until they had accumulated a total of 50 hrs. each. The x-ray plates during this time were taken during rest periods so as to determine the mean size of the heart during rest. The treadmill was elevated at an angle of 14° and was run by a small electro motor. Its speed during these preliminary studies was 7,200 feet per hour; during the first set of experiments, it was increased to 9,900 ft. per hour and in the second set of experiments to 17,250 ft. per hour.

A setter (pericardiotomized dog) weighing 38 lbs. was put on the treadmill and run steadily at speed of 9,900 ft. per hour for 7 hrs., thereby performing 657,282 foot lbs. work. At the end of 7 hrs. the dog seemed quite fatigued and refused to run. Pulse at beginning was 126 and at the end of exercise 172 and very rapidly returned to the normal, 102. The dog was x-rayed at once.

Mean value of silhouette area of pericardiotomized dog as measured over a long period of time = 69.9 ± 3.5 sq. cm. After 7 hrs. running = 65.7. Difference, 4.2 sq. cm.

A hound, weight 39 lbs. (control) was run for exactly 7 hrs. under the same conditions, thereby performing 674,053 foot lbs. work. At the end of 7 hrs. the control dog seemed very tired. Pulse before running was 114 and after running 168. Returned to normal at once. He was x-rayed immediately.

Mean value of silhouette area of control dog as measured over a

long period of time = 69.4 ± 3.5 sq. cm. After 7 hrs. running = 69.3.

In another set of experiments, the speed of the mill was increased to 17,250 ft. per hour. The same dogs were used as in the previous experiment. The dogs were run for 3 hours each.

Pericardiotomized dog—

Immediately before running	72.0 sq. cm.	Mean value of silhouette
Immediately after running	66.6 " "	area over a long period
		of time = 69.9 ± 3.5.
Decrease in size of	5.4	

Control dog—

Immediately before running	71.8 " "	Mean value of silhouette
Immediately after running	67.5 " "	area over a long period
		of time = 69.4 ± 3.5.
Decrease in size of	4.3	

In this experiment the size of both the heart of the pericardiotomized dog and of the control dog decreased in size following forced severe exercise. This decrease in size was greater than the limit of error. Our results are in accordance with most of the work done heretofore on the effect of exercise upon the size of the heart. The pericardiotomized animal reacted to exercise in exactly the same manner as a normal animal, *i. e.*, a slight decrease in heart size.

The dogs were in no way affected by the removal of the pericardium.

These experiments are in agreement with Stewart,[1] Yamada,[2] Beck and Moore.[3]

4806

The Point of Origin of the Bronchial Breath Sounds.

GEORGE FAHR AND JAY DAVIS.

From the Department of Medicine of the University of Minnesota and the Minneapolis General Hospital.

A tracheotomy was performed on a patient who had a carcinoma of the larynx, and a pneumonia developed in the left lower lobe. With the patient's nose closed firmly by the fingers of the doctor

[1] Stewart, *J. Clin. Invest.*, 1929, 339.

[2] Yamada, *Mitt. a. d. Med. Falc. d. k. Univ. zu Tolcio*, 1917, xvi, 527.

[3] Beck and Moore, *Arch. Surg.*, 1925, xi, 550.

and the mouth closed firmly by the patient, bronchial breath sounds which sounded typical were heard over the left lower lobe. The breath sounds over the other lower lobe were normal. This would seem to show that the vocal cords play no very large rôle in the formation of the bronchial breath sounds, but that the bronchial breath sounds are formed by the passage of air through the bronchial tubes much after the manner of the formation of sound in lip pipes.

A patient who had loud bronchial breath sounds over the right lower lobe came to autopsy. A rubber tube was passed through the vocal cords and air was periodically allowed to enter the lungs from an oxygen tank. There was a side tube for allowing the egress of air and by alternately closing the outflow and inflow tubes, artificial inspiration and expiration could be produced. Auscultation over the right lower lobe produced bronchial breath sounds resembling fairly closely those heard over the same area by the intern and resident physician before the death of the patient. The trachea was now cut off below the larynx and the same rubber tube was inserted down just beyond the bifurcation. On auscultation the breath sounds were still bronchial and apparently were a little higher pitched than when the tube was passed just beyond the vocal cords. This experiment shows that the components of the bronchial breath sounds are formed largely in the bronchial tubes below the vocal cords and that some of the bronchial breath sound is formed apparently beyond the primary bronchus.

Conclusions: The vocal cords play only a very small part in the formation of the bronchial breath sounds. Bronchial breath sounds are not formed on the principle of the reed pipe; more probably on the principle of the lip pipe.

4807

Relations Between Surface Area, Weight and Length of the Human Body in Prenatal Life.

ALBERT D. KLEIN AND RICHARD E. SCAMMON.

From the Department of Anatomy, University of Minnesota.

The growth in surface area of the body in prenatal life has received little study. Valentine,[1] Ssytscheff,[2] Lissauer,[3] Kastner,[4] and Pfaundler[5] have included some determinations of the surface area of premature infants among their observations but most of these

were of children who had lived several days or weeks after birth. Sandiford[6] has estimated the surface in prenatal life by the application of the formulae of Lissauer, and of Du Bois to the data of Mall on the length and Jackson on the weight of the fetus at various ages.

We have determined the surface area of the body in a series of 12 fetuses ranging from 2.68 to 31.98 cm. in crown rump length and from 1.26 to 2463.00 gm. in weight. All specimens but one (the largest) were preserved in formalin prior to measurement. The largest specimen was a fresh cadaver and the measurement of its surface was made from a plaster of Paris cast of the body. Our method was as follows: The specimen was first covered with 2 thin coats of lacquer by dipping it in a lacquer solution. When the second lacquer coat was almost dry the specimen was dipped in a hot mixture of water (15 parts by volume), gelatin (5 parts by weight), and glycerin (1 part by volume). This coat hardened into a pliant but non-elastic film which was removed and cut into small pieces. The outlines of these pieces were traced on celluloid and their areas determined from weight by the methods described by Boyd and Scammon,[7] and Scammon and Scott.[8] This method was used for

TABLE I.—Surface area in prenatal life.

Specimen number and sex	Crown-rump length (cm.)	Crown-heel length* (cm.)	Weight† (gm.)	Observed surface area (sq. cm.)	Surface area (sq. cm.) Calculated by formula: (1)	(2)	(3)
1 (m)	2.68	3.27	1.26	5.82	4.17	4.85	6.17
2 (m)	6.02	8.28	11.33	32.37	34.64	33.83	32.04
3 (m)	6.64	9.21	16.15	47.22	44.15	42.81	41.79
4 (m)	10.36	14.79	72.17	129.66	129.88	124.57	128.47
5 (f)	15.32	22.23	221.00	277.70	328.60	318.66	297.34
6 (f)	18.04	26.31	348.50	421.57	482.36	471.79	418.42
7 (f)	18.63	27.20	462.00	602.86	520.12	509.68	516.94
8 (m)	21.14	30.96	664.00	715.90	698.84	690.37	678.57
9 (m)	23.51	34.52	937.20	854.42	895.16	891.02	878.68
10 (f)	26.69	39.29	1428.20	1083.67	1202.20	1208.30	1205.10
11 (m)	29.17	43.01	2075.00	1510.27	1477.40	1495.70	1594.90
12 (f)	31.98	47.22	2463.00	1759.72	1828.20	1865.30	1813.70

*Calculated from crown-rump by empirical formula
$CR = 0.66CH + 0.5$ mm.
†After preservation in formalin.

[1] Valentine, *Lehrbuch der Physiologie des Menschen.* Braunschweig, 1851.
[2] Ssytscheff, A. I., *Diss.*, St. Petersburg, 1902.
[3] Lissauer, W., *Jhrb. f. Kinderheilk.*, 1903, lviii, 392.
[4] Kastner, O., *Z. f. Kinderheilk.*, 1913, iii, 391.
[5] Pfaundler, M., *Z. f. Kinderheilk.*, 1916, xiv, 48.
[6] Sandiford, I., *J. Biol. Chem.*, 1924, lxii, 323.
[7] Boyd, E., and Scammon, R. E., *Anat. Rec.*, 1927, xxxv, 5.
[8] Scammon, R. E., and Scott, G. H., *Anat. Rec.*, 1927, xxxv, 269.

all parts of the body except the fingers and toes. These digital areas were computed geometrically from their lengths and diameters. The major dimensions and observed surface areas of these specimens are shown in the first columns of Table I.

Considering the body as an approximation to a simple geometric form, the relation of its surface area to its length is written:

$$S = aL^b, \text{ or, } \log S = \log a + \log L \cdot b$$

where a and b are constants, the latter of which is expected to approach the second power. A curve, fitted by the method of averages, to our twelve observations gives the formula:

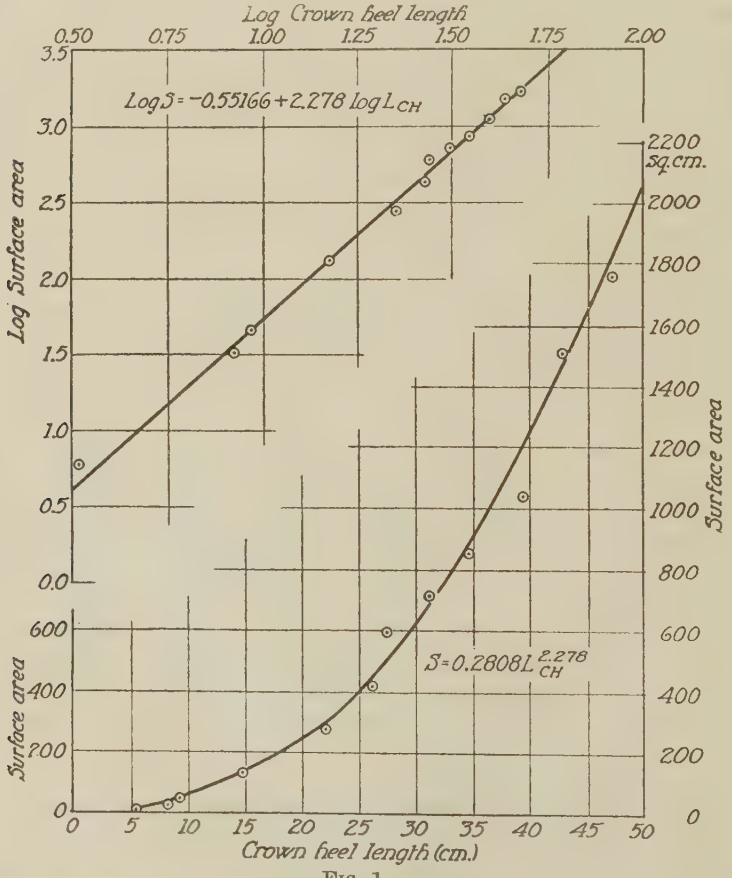

FIG. 1.

Crown heel length and surface area in prenatal life. Upper graph shows relation of logarithms of surface area to logarithms of total or crown heel length. Lower graph shows relation of surface area to crown heel length. Individual observations represented by circled dots. Analytic expressions represented by solid lines.

$$S = 0.2808 L_{ch}^{2.278} \quad (1)$$

The mean absolute deviation of the observed from the calculated values obtained from this expression is 39.86 sq. cm. and the mean relative deviation is 9.4%. (See Fig. 1.)

Applying the same analysis to surface and crown rump length we obtain the expression:

$$S = 0.4545 L_{cr}^{2.401} \quad (2)$$

The mean absolute deviation of the observed from the calculated values is 41.94 sq. cm. and the mean relative deviation is 8.7%. (See Fig. 2.)

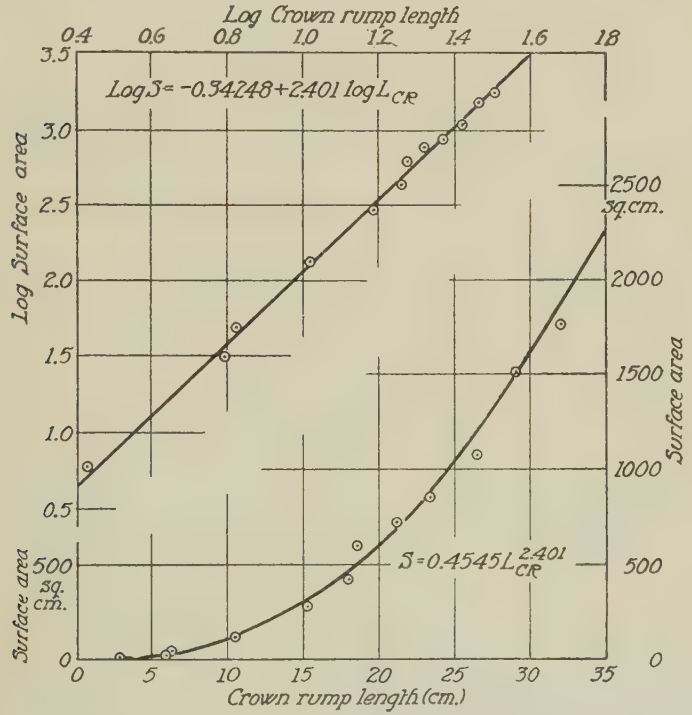

FIG. 2.

Crown rump length and surface area in prenatal life. Lower graph shows relation of the logarithms of surface area to logarithms of crown rump length or sitting height. Lower graph shows relation of surface area to crown rump length.

Similarly, from geometrical considerations, the expression for the relation of surface to weight would be:

$$S = aW^b, \text{ or, } \log S = \log a + \log W \cdot b,$$

the constant b approximating the 2/3 or 0.667 power.

Fitting a curve to the observations by the method of averages, we obtain

$$S = 5.188W^{0.750} \quad (3)$$

where S is the surface in square centimeters and W is the weight of the body in grams. The mean absolute deviation of the calculated from the observed values is 36.39 sq. cm. and the mean relative deviation is 5.8%. (See Fig. 3.)

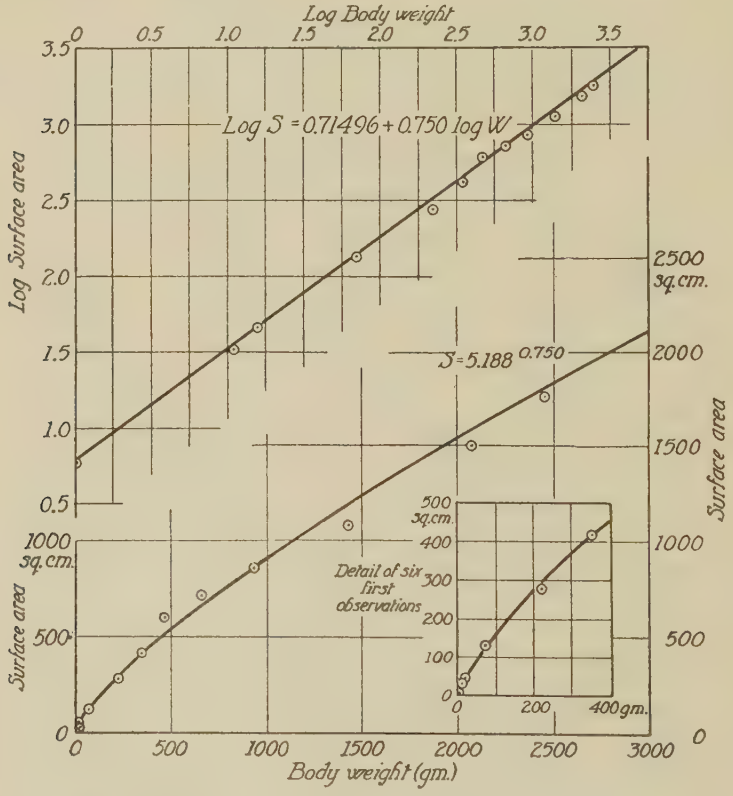

FIG. 3.

Body weight and surface area in prenatal life. Upper graph shows relation of logarithms of surface area to logarithms of body weight. Lower graph shows relation of surface area to body weight.

We have also applied the classic weight-length formula of Du Bois and Du Bois[9] to these data. The mean absolute deviation of calculated from observed values is 40.37 sq. cm. and the mean relative deviation is 21.3%. But it is obviously uncritical to adjudge the Du Bois' formula, which was developed for adults of diverse build by its application to the human body in its early stages of growth.

[9] Du Bois, D., and Du Bois, E. F., *Arch. Int. Med.*, 1916, xvii, 863.

We have, therefore, developed new expressions involving height and weight, using the Du Bois method of determining constants. We have obtained a number of expressions showing about equal measures of goodness of fit. Among the best are:

$$S = 113.48 \cdot W^{13/27} \cdot H^{5/9} \quad (4)$$
$$S = 452.40 \cdot W^{11/18} \cdot H^{1/6} \quad (5)$$

The mean absolute deviation of the calculated values by (4) from the corresponding observed surfaces is 71.63 sq. cm. and the mean relative deviation is 15.4%. The corresponding deviations of (5) are 70.2 sq. cm. and 15.8%.

4808

Surface Area and Age in Prenatal Life.

RICHARD E. SCAMMON AND ALBERT D. KLEIN.

From the Department of Anatomy, University of Minnesota.

In a preceding paper we have given a series of empirical formulae for the relation between the surface area and some of the major dimensions of the human body in prenatal life. These formulae are as follows:

$$S = 0.2808 L_{ch}^{2.278} \quad (1)$$
$$S = 0.4545 L_{cr}^{2.401} \quad (2)$$
$$S = 5.188 W^{0.750} \quad (3)$$

In these expressions S is the surface area of the body in square centimeters, L_{ch} is the total or crown heel length in centimeters, L_{cr} is the sitting height or crown rump length, and W is body weight in grams.

The increase in surface area with respect to age in the fetal period may be estimated by the substitution of expressions for time in terms of body length or body weight in this period. We have done this using the empirical formulae of Scammon and Calkins.[1, 2] In these expressions age is given in lunar months (of 28 days) dated from the first day of the last menstruation. These expressions only hold for the fetal period proper (from 3 lunar months to birth).

[1] Scammon, R. E., and Calkins, L. A., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 253.

[2] Scammon, R. E., and Calkins, L. A., PROC. SOC. EXP. BIOL. AND MED., 1924, xxii, 157.

The accompanying table gives the calculated values for the surface area of the body at each month of the fetal period, as computed through crown heel length, through crown rump length and

TABLE I.—Calculated Surface Area in the Fetal Period.

Age (lunar months)	Surface area (sq. cm.) as calculated from:			Derivatives [Calculated from (1)]		
	(1) Crown heel length	(2) Crown rump length	(3) Body weight	Velocity (sq. cm. per mo.)	Relative velocity (% per mo.)	Acceleration (sq. cm. per mo. per mo.)
3	24.3	23.5	38.1	72.7	299.5	106.1
4	145.7	136.7	147.5	165.4	113.5	80.0
5	347.8	329.9	336.7	235.8	67.5	62.0
6	612.5	588.8	590.4	291.5	47.6	50.1
7	927.5	902.6	902.6	337.3	36.4	41.9
8	1284.5	1263.6	1262.3	375.9	29.3	35.8
9	1677.6	1666.2	1664.2	409.4	24.4	31.3
10 (birth)	2102.0	2105.4	2102.2	438.8	20.9	27.7

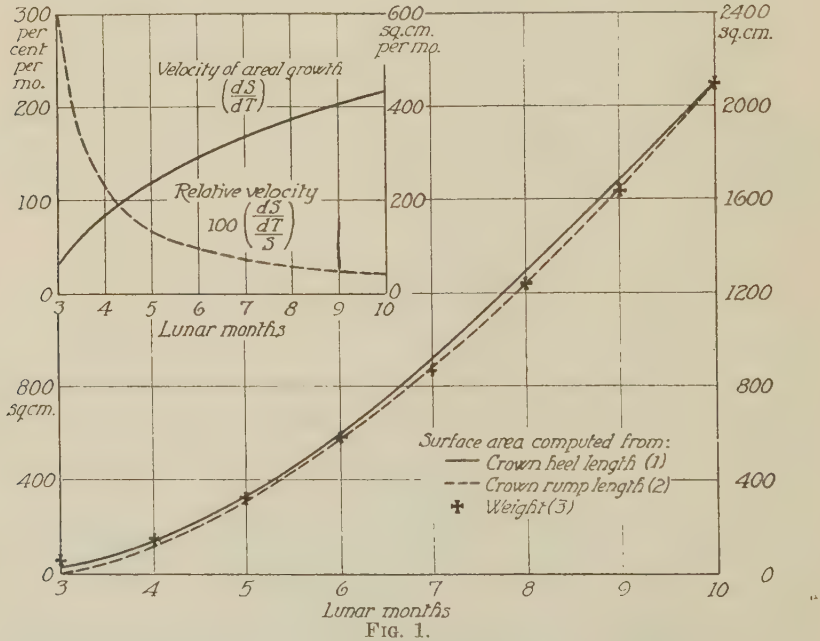

FIG. 1.

Graphs illustrating the growth of the surface area of the body, with respect to age, in the fetal period. The major graph shows the course of growth in surface area as computed from (1) crown heel or total length, (2) crown rump length or sitting height and (3) body weight. The solid line in the minor graph shows the velocity of growth (in square centimeters per month) of surface area as computed from crown heel length. The curve drawn in broken line shows the relative velocity of growth (first differential times 100, divided by attained magnitude) of surface area as computed from crown heel or total body length.

through body weight. The agreement of these figures is quite close —particularly for crown rump length and body weight, with the exception of the values at 3 lunar months.

We have also calculated the velocity, the relative or percentage velocity and the acceleration of areal growth in this period (as estimated through crown heel length). These values are shown in the last 3 columns of the table. The accompanying figure shows the results of these computations in graphic form.

4809

The Regional Growth in Surface Area of the Human Body in Prenatal Life.

ALBERT D. KLEIN AND RICHARD E. SCAMMON.

From the Department of Anatomy, University of Minnesota.

The surface areas of the chief regions of the body were determined for 12 fetuses ranging from 3.27 to 47.22 cm. in total or crown heel length and from 1.26 to 2463.0 gm. in weight. The details of the material and method employed are described in a preceding paper.

The regions delimitated were head, neck and trunk (including the perineal region and the penis and scrotum in the males), the upper extremities (both sides), and the lower extremities (both sides including gluteal regions). From geometrical considerations, that were found to be applicable to measurements of the surface area of the body as a whole, it was thought that an adequate expression for representing the relation of the surface area of a part to body length might be:

$$S_p = aL^b, \text{ or, } \log S_p = \log a + \log L \cdot b$$

where S_p is the area of the part in question, L is the total or crown heel length of the body and b is an exponent approaching 2. Graphic tests on double logarithmic paper indicated that this surmise was justified.

When fitted by the method of averages the following expressions were obtained:

$$S_h = 0.1767L^{1.997} \quad (1)$$
$$S_t = 0.1191L^{2.207} \quad (2)$$
$$S_u = 0.0244L^{2.449} \quad (3)$$
$$S_l = 0.0216L^{2.632} \quad (4)$$

where S_h is the area of the head, S_t is the area of the trunk, S_u is the area of the upper extremities and S_l is the area of the lower extremities.

The mean absolute deviation of the observed from the calculated values by formula (1) is 12.2 sq. cm. and the mean relative deviation is 9.9%. Omitting the first observation, (on a specimen 3.27 cm. in length) the mean relative deviation is 8.4%. The mean deviation (taken without regard to sign) of the observed from the calculated values given by formula (2) is 21.0 sq. cm. and the mean relative deviation is 13.2%, or omitting the first observation, 12.8%. The mean absolute deviation (taken without regard to sign) of the observed from calculated values by formula (3) is 12.1 sq. cm. and the mean relative deviation is 12.2%, or omitting the first observation,

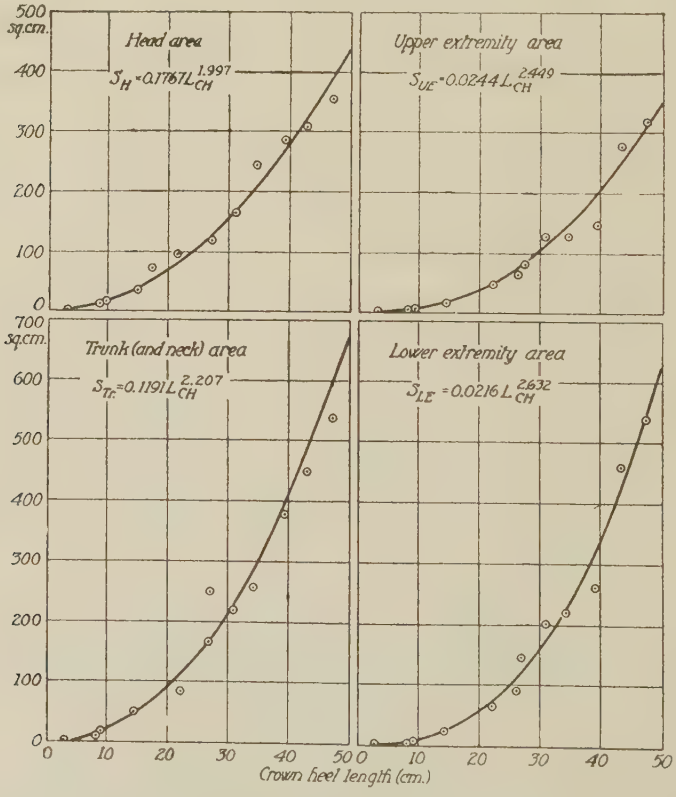

FIG. 1.

Graphs showing relationships of the areas of various regions of the body to the total or crown heel length. Abscissae, crown heel length in centimeters; ordinates, areas of the major parts of the body in square centimeters. The individual observations are indicated by circled dots. The curves are drawn to the several formulae given in the graphs.

11.0%. The mean absolute deviation (taken without regard to sign) of the observed from the calculated values by formula (4) is 17.3 sq. cm. and the mean relative deviation is 13.8%, or omitting the first observation, 12.3%. These deviations, while high, are not surprisingly so, considering the difficulty of the technique involved in determining the areas of such small objects and the uncertainty of obtaining exactly the same delimitations of regions of the body in all specimens. Fig. 1 shows the observations and the corresponding fitted expressions in graphic form.

It will be noted that the exponents of these formulae for areas of regions form an ascending series starting somewhat below the second power and ending between the second and third power. Consequently the relative velocities of growth of the several areas are in series, the lower extremities growing the most rapidly, with the rates decreasing in succession for upper extremities, trunk and head. Thus the growth in surface areas of the major regions of the body,

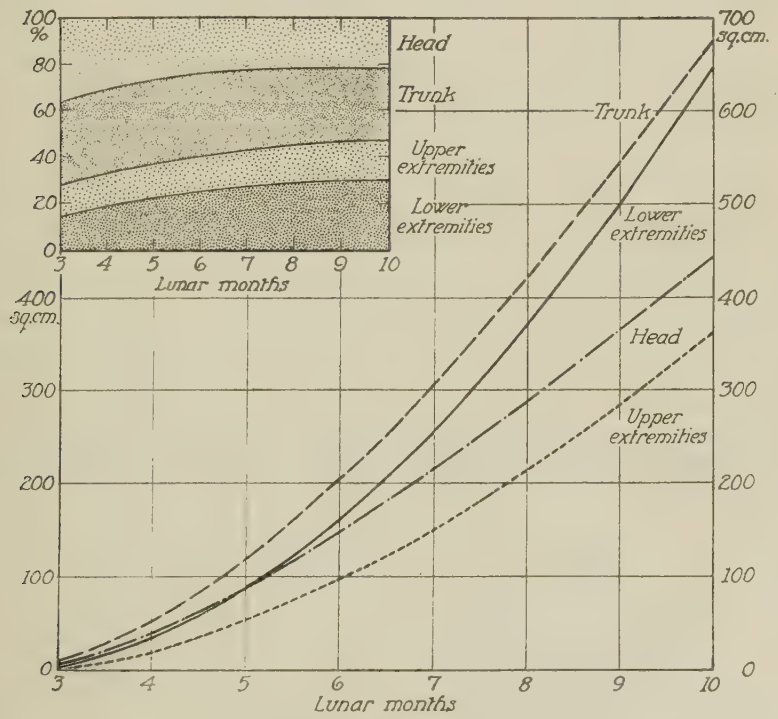

FIG. 2.

Major graph: curves of growth in calculated area of the major parts of the body in the fetal period. Abscissa, age in fetal or lunar months; ordinates, surface areas of the major regions of the body in square centimeters. Minor graph: a histogram illustrating the changes in relative area of the major parts of the body in the fetal period.

like their volumes and dimensions (as shown by Calkins and Scammon,[1] Scammon,[2] and Scammon and Calkins,[3]) follow very definitely the sequence known as the law of developmental direction.

The main portion of Fig. 2 shows the computed curves of growth in area of these several regions of the body. They have been placed on a time basis by computing menstrual age from body length by the empirical formula of Scammon and Calkins.[4]

TABLE I.

Calculated per cent of total surface area formed by the surface areas of the several regions of the body in each month of the fetal period. (Values given to the nearest 0.1 %.)

Age (lunar months)	Total surface area* (sq. cm.)	Percentage surface area of:			
		Head	Trunk	Upper extremities	Lower extremities
3	24.46	36.0	36.6	12.1	15.2
4	143.05	29.6	35.6	14.2	20.7
5	341.53	26.6	34.6	15.1	23.7
6	603.98	24.7	33.9	15.7	25.7
7	919.56	23.4	33.2	16.1	27.3
8	1280.61	22.3	32.7	16.4	28.5
9	1681.68	21.5	32.3	16.7	29.6
10	2118.20	20.8	31.9	16.9	30.5

* Sum of calculated values for parts.

The calculated areas of the regions of the body computed as per cents of the combined calculated surfaces are shown in Table I and the upper panel of Fig. 2 presents the same results in graphic form. They show, in a more tangible way, the seriation of areal growth indicated by the empirical formulae.

[1] Calkins, L. A., and Scammon, R. E., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 353.

[2] Scammon, R. E., PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 238.

[3] Scammon, R. E., and Calkins, L. A., "Development and growth of the external dimensions of the human body in the fetal period." Minneapolis, 1929.

[4] Scammon, R. E., and Calkins, L. A., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 353.

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Effects of Winter Solar Irradiation and of Cod Liver Oil on Production and Fertility of Eggs.

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In early November, 1927, a small colony of hens and cockerels from a flock of white leghorns of uniformly good grade of stock which had summered out of doors were divided into 3 groups of 6 hens and one cockerel each. They were placed in an experimental house, each compartment of which was provided with transparent windows of about 10 square feet, with southern exposure to light. One compartment was provided with cathedral or hammered vitaglass, while the other 2 were fitted with cathedral ordinary window glass. The diet fed throughout the experiment was the standard Wisconsin all-mash ration, consisting of 80 pounds yellow corn meal, 20 pounds shorts, 5 pounds bone meal, 5 pounds limestone grit and 1 pound salt. Group 1 was enclosed behind ordinary window glass with 2% by weight of cod liver oil (Squibb's) added to the diet, group 2 behind quartz-containing glass (vitaglass) and group 3 behind ordinary window glass only. The hours of feeding were regular and the quantities of food given were ample but constant. Temperature, amount of sunshine and other elements of the weather changed from day to day but were the same in all compartments. No attempt was made to keep the temperature in the experimental chicken house within a certain range; during 6 months of experimentation the temperature varied from 60° F. to —20° F. In brief, the experimental conditions were similar to those existing on a Minnesota farm; the only variables controlled were the presence or absence of the shorter ultraviolet rays of winter sunlight or cod liver oil in the ration.

Starting in January, 1928, all eggs from each of the 3 groups were gathered, and incubation of the weekly product was made.

Production of Eggs. The total gathering of eggs each week from all compartments and the percentage of production under each of the 3 experimental conditions, are given in the tabulation.

Curve 1 of Fig. 1 shows the production under ordinary window glass and 2% by weight of cod liver oil added to the diet. The point *A* indicates the value of the average percentage production over period of the experiment. The line *a b*, indicates the average rate of

TABLE I.

Influence of presence or absence of the ultraviolet region (290 to 320 millimicrons) of winter sunlight or of cod liver oil in the ration.*

Date, 1926	Eggs total	Production, %			Fertility, %		
		Vita-glass	Ordinary glass	Ordinary glass and cod liver oil (2% by wgt.)	Vita-glass	Ordinary glass	Ordinary glass and cod liver oil (2% by wgt.)
1-18	32	27	33	40	100	100	85
1-25	36	31	28	41	80	70	80
2- 1	40	33	22	45	54	55	61
2- 8	33	35	21	54	75	60	77
2-15	37	33	29	38	75	80	85
2-22	29	39	20	41	55	17	40
3- 2	29	39	20	41	55	16	75
3- 9	33	35	24	40	75	50	54
3-16	44	44	11	45	95	40	85
3-23	36	40	20	40	33	0	70
3-30	35	43	11	46	80	25	94
4- 6	38	47	13	40	77	40	73
4-13	36	30	12	58	55	50	81
4-20	35	23	17	60	100	66	86
Average		36	20	44	72	47	75

* All the hens were fed the Wisconsin standard ration and were kept under identical conditions of warmth, ability to exercise and the like. The only variable factor, as far as is known, was the character of the spectral filter used or the addition of cod liver oil to the ration.

increase in production of eggs. Curve 2 shows the results with exposure under vitaglass, and curve 3 gives the results obtained with ordinary window glass, the standard Wisconsin ration being fed. The significance of the points *B* and *C* and the lines *c d* and *e f* are analogous to those made with reference to point *A* and line *a b* in connection with curve 1.

These data and curves show that the greatest production of eggs is under ordinary glass with the addition of 2% cod liver oil to the diet. Next in order, and approximating during the latter part of March the production in Group 1, is the production under vitaglass with the standard Wisconsin ration. The least production is in Group 3, under ordinary glass only. The slopes of the lines *a b*, *c d* and *e f* indicate that production is increased from January to May under vitaglass or ordinary glass and cod liver oil, but that the production is decreased at a fairly constant rate under ordinary glass only.

Fertility of Eggs. The tabulation gives a record of the number of eggs incubated and the percentage of fertility in the 3 groups. The weekly product was incubated for one week only; the shells were opened and the eggs classified as fertile or nonfertile. We did

not carry any of these incubations to the point of hatching. Curve 1 of Fig. 2 shows the fertility under ordinary window glass and 2% cod liver oil; curve 2, the fertility under vitaglass, and curve 3, the fertility under ordinary window glass, the standard Wisconsin ration being fed in all cases. It is noted that the curves of percentage fertility for the 3 groups are of the same type and practically coincide during the months of January and February. From February

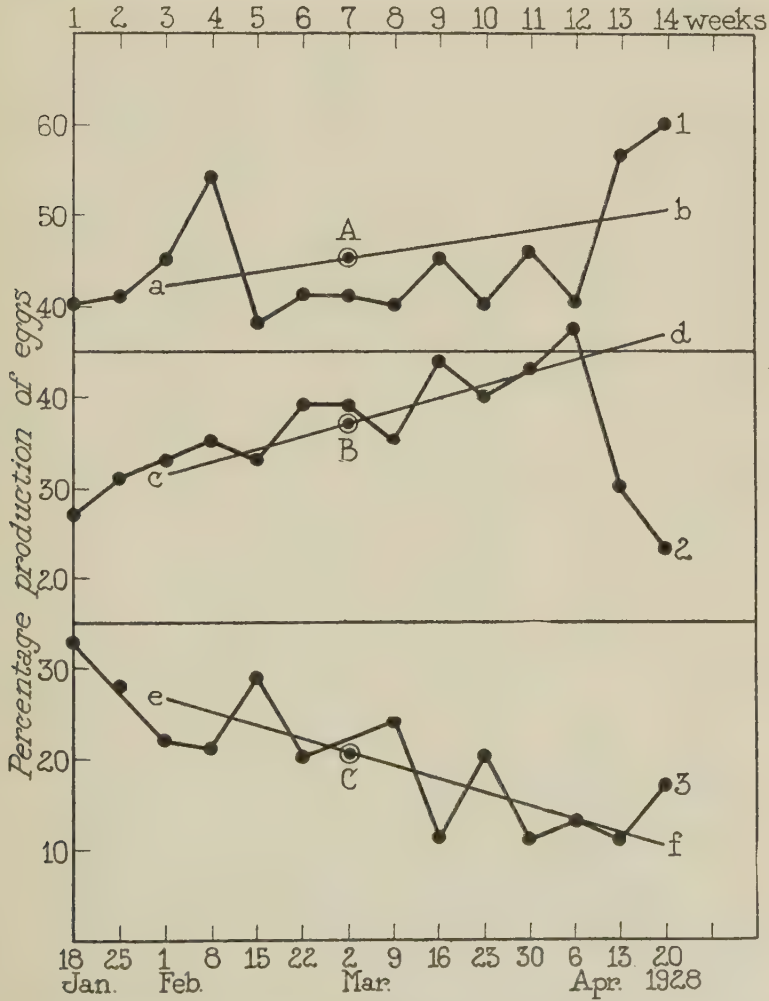

FIG. 1.

Weekly changes in percentage production of eggs during the winter months; curve 1, percentage production under ordinary window glass and 2% by weight of cod liver oil added to the diet; curve 2, under quartz-containing glass (vitaglass), and curve 3, under ordinary window glass.

15 on, however, the curves of percentage fertility of eggs are radically different. The lines *a a* and *a b* show the approximate average rate of increase of fertility when the stock is kept behind ordinary glass with cod liver oil in the diet and behind vitaglass respectively. The line *a c* curve 3, indicates that the rate of fertility of eggs under

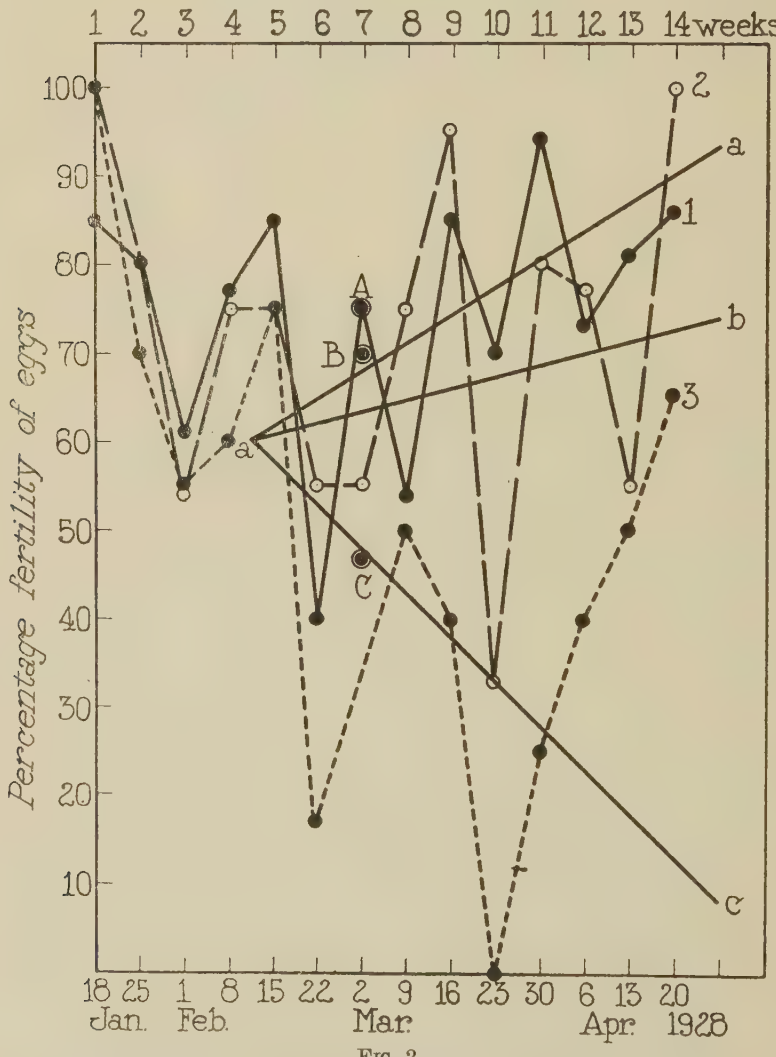

FIG. 2.

Percentage fertility of eggs during the winter months as affected by cod liver oil and winter solar irradiation; curve 1 shows the percentage fertility of the total production of eggs under ordinary window glass and 2% cod liver oil added to the standard Wisconsin ration; curve 2, under quartz-containing glass (vitaglass), and curve 3, under ordinary window glass and with the standard Wisconsin ration.

ordinary glass and the standard Wisconsin ration decreases, with, however, a noticeable increase in fertility when the month of April is reached. This marked increase in fertility may be attributed logically to the considerable increase in ultraviolet content of the sunlight during April and to the fact that other untoward physiologic conditions, such as low temperatures, did not exist, as in the midwinter months.

The tabulation contains data showing the average percentage of total production and average percentage fertility of each group during the 14 weeks of the experiment. The average percentages of production and fertility are about the same under vitaglass or ordinary window glass with cod liver oil, and these percentages are practically double the percentages obtained when ordinary glass and the standard ration only are used.

The transmissions of vitaglass and ordinary glass and the ultraviolet content of winter sunlight. Vitaglass transmits about 20% of radiation in the range of so-called vital rays (290 to 320 millimicrons), 70% in the ultraviolet region, 290 to 400 millimicrons, 90% of the visible region, 400 to 750 millimicrons, and 70% of the infra-red incident rays. Ordinary window glass does not differ appreciably from vitaglass in its transmission of the infra-red and visible portions, but transmits only 2% of solar energy in the region 290 to 320 millimicrons and 35% of the total ultraviolet from 290 to 400 millimicrons. The content and energy of the ultraviolet portion of winter sunlight have been measured by several investigators; reports of conditions in Kansas and Maryland were made recently by Hughes and Pycha,[1] and by Clark.[2] Hughes and Pycha, in their measurement of vita rays (rays which have the special property of regulating calcium and phosphorus metabolism in the animal organism), used the acetone methylene-blue method and concluded that there was an average daily reading of 1.1 unit during January, 1.4 unit during February, and 1.3 unit during March. In young growing chickens kept back of celoglass (which transmits 35 to 40% of the vita radiation of the sunlight) and which, therefore, received less than half a unit of irradiation a day rickets did not develop, whereas in control group of chickens behind ordinary window glass rickets developed in from 6 to 12 weeks. Clark found the average ultraviolet content of sunlight in Baltimore during January to be 1.2 methylene-blue units, during February 1.7 units, and during

[1] Hughes, J. S., and Pycha, R. L., *Tr. Illuminating Engineering Soc.*, 1928, xxiii, 233.

[2] Clark, Janet H., *Am. J. Hyg.*, 1929, ix, 646.

March, 2.3 units. Our own determinations, although not made as methodically or as intensively as those of Clark or Hughes and Pycha, showed that the average ultraviolet content of sunlight in Rochester, Minnesota (away from smoke and in the open country) were 1.2, 1.4 and 1.3 methylene-blue units during January, February and March, respectively. Since the vitaglass, by reason of solarization and films deposited by weather and other conditions, did not transmit more than 50% of the total ultraviolet content of sunlight, we may conclude that the average ultraviolet received by stock placed behind vitaglass was not in excess of half of a methylene-blue unit. The reception of solar radiation in the region 290 to 310 millimicrons did not exceed 15% and the intensity of solar radiation in this range of wavelengths amounted to about 100 ergs for each square centimeter each second. Clark stated that, to get the maximal antirachitic effect, the daily length of exposure multiplied by the methylene-blue units must equal 2.5. Since the vitaglass transmits on the average half a unit, it appears that the ultraviolet content of the winter sunlight for a daily period of 6 to 8 hours is ample to maintain normalcy, so far as the antirachitic factor is concerned. Other observations on the antirachitic effect of December sunlight in Toronto have been made by Tisdall and Brown,[3, 4] and by Fleming[5] regarding winter sunlight in Washington. Tisdall and Brown concluded that the rays from December sunlight which have passed through vitaglass had an antirachitic effect which is roughly one-fourth of the value of the direct rays, whereas the rays through ordinary glass had no rachitic properties. Fleming concluded that normal bone calcification was produced in the rats under vitaglass during the winter months in Washington.

There is apparently a close parallelism between the effects of ultraviolet light or the content of vitamine D in cod liver oil in the prevention of rickets in chickens and the efficacy of these agents in either producing increase in, or maintaining normalcy of, production and fertility of eggs, provided a diet rich in mineral content and accessory vitamines (other than vitamine D) is fed. The production of eggs is greatly influenced by the presence of the antirachitic factor in the diet or the environment even though there is plenty of lime in the ration. The irradiation of hens with such ultraviolet

[3] Tisdall, F. F., and Brown, Alan, PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 446.

[4] Tisdall, F. F., and Brown, Alan, PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 449.

[5] Fleming, W. D., *Mil. Surgeon*, 1928, lxii, 592.

light as passes from winter sunlight through quartz glass, or the presence of cod liver oil in the diet, markedly increases the amount of lime in the shell as compared with the eggs from hens kept on the standard Wisconsin ration under ordinary window glass. Furthermore, Hart, Steenbock,[6] and their collaborators showed that the feeding of cod liver oil, or the irradiation of hens with ultraviolet light (Cooper-Hewitt quartz lamp) after a long confinement to a rachitic ration with resultant decrease in production of eggs again stimulates and increases production of eggs, and that irradiation with ultraviolet light improves the hatchability of the eggs. Hughes, Titus and Moore[7] found that the percentage of eggs hatched from the layings of hens exposed to direct sunlight or to irradiation by mercury quartz lamps was of the order of 75% and that the hatchability in a control group housed behind ordinary window glass dropped to 53%. They also stated that cod liver oil at the rate of 0.5 cc. for each hen daily produced eggs which hatched as well as those from hens receiving direct sunlight.

The results of our experiments and those of other investigators indicate 2 practical conclusions: (1) those who use fowls as experimental animals and who are desirous of maintaining normal stock, production of eggs, fertility and hatchability without the use of quartz-mercury lamps during the winter months in the north temperate zone may do so by the installation of mediums reasonably transparent to the ultraviolet region of sunlight from southern exposures or by the addition of cod liver oil to the diet and (2) to the poultryman who is desirous of obtaining the same results, it is probable that the use of cod liver oil will prove adequate and sufficiently inexpensive to warrant its use.

Conclusions: 1. Sufficient ultraviolet light is transmitted by such quartz-containing glasses as vitaglass or helioglass in the winter months in Minnesota to keep production and fertility of eggs at a high level.

2. Ordinary window glass screens out irradiations from winter sunlight which are beneficial to the maintenance of high production and fertility of eggs.

3. It appears that these results are due either to the transmission of solar energy in the region 290 to 320 millimicrons or to the greater transmission of the ultraviolet (320 to 400 millimicrons) of sunlight by quartz-containing glass.

[6] Hart, E. B., Steenbock, H., Lepkovsky, S., Kletzien, S. W. F., Halpin, J. G., and Johnson, O. N., *J. Biol. Chem.*, 1926, lxv, 579.

[7] Hughes, J. S., Titus, R. W., and Moore, J. M., *Science*, 1925, lxii, 492.

4. The addition of 2% cod liver oil to the standard Wisconsin ration gives results the equal of or slightly superior to those obtained with winter sunlight passed through an ultraviolet transmitting medium.

5. From our experiments and those of other investigators there is evidence that the presence or absence of the antirachitic factor vitally affects the production, fertility and hatchability of eggs.